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TIME-TEMPERATURE EFFECTS ON SALMONELLAE
AND STAPHYLOCOCCI IN FOODS: II. BEHAVIOR
AT WARM HOLDING TEMPERATURES THERMAL-
DEATH-TIME STUDIES

Robert Angelotti, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1960

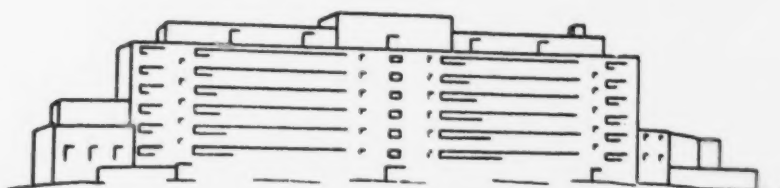
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II. Behavior at Warm Holding Temperatures. Thermal-Death-Time Studies.



The Robert A. Taft
Sanitary Engineering Center

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TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

II. Behavior at Warm Holding Temperatures. Thermal-Death-Time Studies.

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Milk and Food Research Program

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

ABSTRACT

Food poisoning is more prevalent in the United States than is generally recognized. Though official reports list approximately 10,000 cases per year, it is generally estimated that ten to fifty times this number actually occur. The most commonly reported types of foodborne disease outbreaks are caused by salmonellae and staphylococci. A practical method of preventing foodborne disease outbreaks is the application of temperatures that will inhibit or destroy the causative organisms in perishable foods. Toward this end, studies were undertaken to establish experimentally the temperature limits for growth of salmonellae and staphylococci in ham salad, chicken à la king, and custard in the range of 95°F to 120°F. In addition, thermal-death-time studies were conducted on salmonellae and staphylococci in the same foods at 5°F intervals from 130°F to 150°F.

Mixed cultures of Salmonella senftenberg 775W, S. enteritidis, S. manhattan, and Staphylococcus aureus 196E, Staph. aureus Ms149, and Staph. aureus MF159 were cultured in the three foods for 24 hours at 95°F and at two-degree intervals from 112°F to 120°F. In custard, both groups of organisms grew at 114°F, but decreased in numbers at 116°F through 120°F. In chicken à la king, the staphylococci grew at 112°F and were killed at all the higher temperatures. The salmonellae grew at 114°F, but were killed at 116°F and above. In ham salad, both groups of organisms decreased in numbers at temperatures of 112°F through 120°F. Good growth of the test organisms in all three foods was observed at 95°F.

The results of this study and that reported earlier* indicate that the temperature growth range for salmonellae and staphylococci in foods of the type studied is 44°F to 114°F; and that holding perishable foods in the mid-portion of this range for periods that permit growth of salmonellae and staphylococci is to be avoided because of their rapid multiplication.

Thermal-death-time studies conducted with heat-resistant S. senftenberg 775 and Staph. aureus 196E and with non-heat-resistant S. manhattan and Staph. aureus Ms149 yielded the following F₁₄₀ values (number of minutes exposure at 140°F required to reduce the inoculum to a level which is not detectable): S. senftenberg 775W in custard 78 and in chicken à la king 81.5; S. manhattan in custard 19 and in chicken à la king 3.1; Staph. aureus 196E in custard 59 and in chicken à la king 47; Staph. aureus Ms149 in custard 53 and in chicken à la king 40.

The end points of "survival-kill" at all the test temperatures for both salmonellae and staphylococci in ham salad were considerably less than in the other foods studied.

The above findings and the D₁₄₀ values (minutes of exposure at 140°F required to effect a 90% reduction in numbers) indicate that heating perishable foods of the type studied to 150°F and holding every particle of food at this temperature for at least 12 minutes reduces 10 million or fewer salmonellae or staphylococci per gram to levels which are not detectable. The same degree of destruction is achieved in similarly contaminated foods held at 140°F for 78 to 83 minutes.

*Robert A. Taft Sanitary Engineering Center Technical Report F59-2.

INTRODUCTION

Food poisoning is more prevalent in the United States than is generally recognized. Official reports list approximately 10,000 cases per year (Dauer and Davids, 1959), but informed medical authorities estimate that ten to fifty times this number actually occur. The most commonly reported types of foodborne disease outbreaks are caused by salmonellae and staphylococci. Between 5% (Smith and Conant, 1952) and 50% (Dubos, 1948) of the human population are carriers of these organisms respectively and they may contaminate any food that is handled without strict sanitary precautions. Domestic animals, such as dairy cows and poultry also harbor these organisms; therefore, milk, eggs, and meat may be seeded naturally with food-poisoning bacteria. Unless perishable foods are refrigerated properly or otherwise preserved, from the time of harvest to their final preparation for table service, harmful bacteria may grow in them. Investigation of outbreaks usually shows that the incriminated food is heavily contaminated and has been held at temperatures favorable to bacterial multiplication for periods which would allow extensive growth of the causative organism. Although these bacteria may be destroyed by thorough cooking, organisms that survive inadequate heat treatment or are allowed to enter after cooling may grow rapidly if the food is not held below 44°F (Angelotti *et al.*, SEC Technical Report F59-2, 1959.) Thus, time and temperature are interdependent factors which must be carefully controlled to prevent the occurrence of bacterial food poisoning.

The popularity of precooked ready-to-serve foods, the rapid development of catering services, the increased use of vending machines to dispense perishable meals, the growth of the restaurant industry, and the popularity of convenience food services in general have greatly complicated the problem of insuring adequate time-temperature control over perishable products. For this reason, a series of studies has been undertaken to develop experimentally an

organized body of technical data on the critical time-temperature relationships applicable to the safe handling of these foods during processing, storage, transportation, and preparation for serving.

Our previous study (*op.cit.*) demonstrated that the practice of storing perishable foods at a temperature of 50°F for more than one day may result in increases of the microbial population; but, because of the slow microbial growth at this temperature, it is possible to store foods safely for short periods, provided the foods are not grossly contaminated with salmonellae or staphylococci. It was also shown that growth of salmonellae, staphylococci, and *C. botulinum* is prevented in perishable foods when the internal temperature is at or below 42°F.

Part one of the present report describes the response to temperature of salmonellae and staphylococci in foods held at 95°F to 120°F. The results of this study and the preceding one define the limits of the "incubation danger zone" for salmonellae and staphylococci in ham salad, chicken à la king, and custard.

Part two of this report is a study of the thermal resistance of salmonellae and staphylococci in these same foods and presents thermal death time values obtained for each group in the temperature range of 130°F to 150°F at five-degree intervals.

STATEMENT OF THE PROBLEM

Because of the prevalence of staphylococcal food poisoning and foodborne salmonellosis in the United States, a study has been undertaken to determine the ecological factors affecting foodborne disease outbreaks by these organisms. Of the many factors which affect the response of microorganisms to their environment, temperature and time can be controlled to minimize food-poisoning episodes caused by bacte-

ria. Though the effects of time-temperature relationships on the activities of microorganisms have been under investigation for many years and are presently recognized as having a profound effect upon microbial life, application of this basic knowledge toward preventing the growth of bacteria or toxin production in perishable foods has not been as extensive as in the canning or dairy industries. Instead, temperature control as an environmental factor important in food sanitation is applied largely on an empirical basis.

It is the purpose of these studies to present organized technical data, obtained experimentally, which outline critical time-temperature relationships relative to the growth, survival, and death of microorganisms in foods representative of those often implicated in food-borne disease outbreaks.

HISTORICAL REVIEW

Thermal Resistance of Microorganisms

Some bacteria, particularly spore formers, are capable of resisting temperatures many times greater than those required to kill other forms of life, and this unusual resistance determines modern sterilization practices. The effect of heat upon bacteria is dependent on time, temperature, and conditions of exposure. The lethal effects of a given exposure time and temperature may differ with changes in menstruum, pH, moisture, and other conditions. The enzymes and other cellular constituents of bacteria may be inactivated at different rates; therefore, the practical criterion of death is the failure of the microorganism to reproduce under suitable conditions.

Thermal Death Points

Death of bacteria and spores by heat was first studied in terms of the "thermal death point", or the lowest temperature which resulted in no survival after a fixed period of exposure, usually 10 minutes. Hampil (1932), in an exhaustive review, pointed out that various experimental conditions of cellular concentration, pH, age of culture, test method, heating menstruum, and others, effected the thermal resistance of the organisms under study.

Thermal Death Time

In 1920, Bigelow and Esty performed a series of experiments in which a more modern approach to the problem was employed. They were first to use the concept of thermal death time and defined this term as follows: "By thermal death point in relation to time is meant the time at different temperatures necessary to completely destroy a definite concentration of spores in a medium of known hydrogen-ion concentration." Employing known numbers of spores suspended in a specified medium and sealed in glass tubes, they demonstrated that the thermal death time was a function of spore concentration and decreased or increased as the temperature was raised or lowered.

The following year, Bigelow (1921) established the logarithmic nature of thermal-death-time curves and showed that a straight line is obtained when "survival-kill" times were plotted on a logarithmic scale versus temperature on a linear scale.

It was several years after Bigelow's demonstration of the logarithmic relationship between destruction time and temperature before thermal-death-time data were presented in the form of a thermal-death-time curve. Townsend, Esty, and Baselt (1938) adopted the original definitions of Ball (1923, 1928) and established their use with a survival-kill technique.

Ball's mathematical methods revealed that thermal-death-time curves could be characterized by a point and a slope. His reference point was the number of minutes required to destroy organisms at 250°F and was characterized by the symbol F. The slope of the curve was symbolized by z and represented the number of degrees of temperature traversed by the curve in one log cycle.

The conventions proposed by Townsend, Esty, and Baselt for plotting thermal-death-time curves were widely accepted and are generally in use today. They noted that "(a) survival points are positive evidence of viability, and the thermal destruction curve must be above (higher in temperature or longer duration) every survival point; (b) destruction points are indicative, but not positive evidence of death, owing to skips, and the thermal-death-time curve should be beneath as many destruction points as possi-

ble, but still be above all survival points; (c) the slope of the thermal-death-time curve should be parallel to the general trend of the survival and destruction points; and (d) at least three and preferably more temperatures should be used to determine a valid thermal-death-time curve."

The thermal-death-time curve as a measure of thermal resistance implies that there is a time for a given temperature, medium, and cellular concentration at which all the organisms will be killed.

Rate of Death of Bacteria and Spores

In addition to the "survivor-kill" method of studying thermal resistance, other techniques were developed in an effort to determine the rate of bacterial death. Early studies were performed by plating methods to determine the number of organisms originally present and the number of viable organisms remaining after different exposure periods. The rate of bacterial destruction was obtained by plotting survivors against time. The conclusion that the rate of death of microorganisms was of a logarithmic order was shown independently by Chick (1908, 1910) and Madsen and Nyman (1907). The logarithmic order of death of bacteria or spores has been thoroughly reviewed by Rahn (1932, 1943, 1945a, 1945b) and is a generally accepted concept.

However, evidence also exists for a non-logarithmic order of death. The papers of White (1953) Niven, et al. (1954), Kaufman and Andrews (1954), and Collins, et al. (1956), describe survivor curves for vegetative organisms which do not describe a semilogarithmic straight line. The nature of these curves and their interpretation has been reviewed by Schmidt (1957), who points out the errors associated with such curves when any by the straight line portions are used. Schmidt declares that granting the existence of non-logarithmic survival curves in some instances, it still seems that the most satisfactory and practical way to handle problems of thermal resistance is by end-point procedures based on the assumption of a logarithmic type survivor curve. Nevertheless, such calculations will be in error, to the extent that the curve deviates from a straight line, when the data are extrapolated to other conditions and cellular concentrations.

Because of the evidence for and against a logarithmic order of death, doubt arises concerning the method for interpreting thermal resistance data. At present, in those instances where non-logarithmic death appears to occur, it is difficult to establish that such is the case, or whether that the non-logarithmic curve is a function of error in experimental technique. Where non-logarithmic curves are obtained, sufficient evidence should be gathered to show statistically that these curves actually do deviate from a straight line. Where logarithmic curves are obtained, the assumption is made that they are correct, in view of the weight of supporting evidence.

Interpretation of Logarithmic Survival Curves

In plotting survival curves, the number of survivors is plotted on the logarithmic scale versus the exposure time on the linear scale, and the curve may be characterized by the velocity constant K , using the following equation (Schmidt 1957):

$$K = \frac{1}{T} (\log N_0 - \log N)$$

where:

K = a constant, depending upon organism, temperature, substrate and logs to the base 10

T = exposure time in minutes.

N_0 = number of organisms present at beginning of time interval.

N = number of organisms present at end of time interval.

The velocity constant K is usually a very small number and cumbersome to use. Because of this, simpler constants mathematically related to K have been introduced. Katzin, Sandholzer, and Strong (1943) pointed out that a reduction to 10% of the original number gave the equation:

$$K = \frac{1}{T} \log \frac{N_0}{0.1 N_0} \text{ or } K = \frac{1}{T} \text{ or } t = \frac{1}{K}$$

They defined the time at which 10% survivors remained as the "decimal reduction time" or DRT. Ball (1943) substituted Z for DRT, as did

others. However, because of the possible confusion between Z and z (slope of the thermal-death-time curve), Schmidt (1950) proposed that D be used in place of Z or DRT, and the symbol D is now in general usage. D also represents the time required for the survivor curve to pass through one cycle when N is plotted against time.

D may also be calculated from thermal death time determinations when all the data are used. To date, three methods have been developed for this purpose: those of Stumbo (1948) and Stumbo, Murphy, and Cochran (1950), which require averaging D values for each exposure time, and that of Schmidt (1957) based on a bioassay type procedure, in which a cumulative test is constructed from the actual data and the LD₅₀ point at which 50% of the tubes will be sterile is determined. In general the three methods yield agreement within 10% for calculated D values, but Schmidt's method has yielded values significantly different from the others. However, since Schmidt (1957) has reviewed the procedures for calculating D and for determining confidence limits of LD₅₀ from the probability curve, his probability procedure appears to

yield D values of greater precision and will probably replace the others in general application.

The procedures outlined above represent various methods of presenting thermal resistance values for microorganisms. Each has application in given circumstances, depending upon the information the investigator seeks. For practical application, the thermal-death-time concept presents data which show the time required to kill all the organisms in a substance at a given temperature. Values derived in this way, however, are valid only for the specified conditions under study, and changes in experimental or circumstantial conditions yield different thermal-death-time values.

However, by calculating D from multiple-sample, thermal-death-time data, an indication of thermal resistance is obtained which is independent of inoculum size. In presenting data which show the time required to reduce the inoculum to a level which is not detectable (thermal death time) and the time required to achieve 90% destruction (decimal reduction time), a highly useful measure of thermal resistance is obtained.

Part One- BEHAVIOR OF SALMONELLAE AND STAPHYLOCOCCI IN SELECTED FOODS HELD AT 95° THROUGH 120°

PURPOSE

These experiments were performed to determine the response of food-poisoning salmonellae and enterotoxigenic staphylococci in foods held at temperatures in the range of 95°F to 120°F. This report, together with the data presented in earlier studies, establishes the temperature limits for growth of these organisms and defines the limits of the "incubation danger zone" for salmonellae and staphylococci in foods representative of the types associated with food-poisoning outbreaks.

METHODS

Preparation of Cultures

Salmonella senftenberg 775W, S. enteritidis, and S. manhattan and Staphylococcus aureus Ms149, Staph. aureus MF159, and Staph. aureus 196E were selected as test organisms on the basis of their response to temperature as reported previously (Angelotti, *et al.*, 1959). Each of the test cultures was maintained as a stock in Bacto cooked meat medium stored at 68°F. At monthly intervals, the stocks were transferred to fresh tubes of cooked meat medium, incubated at 95°F for 24 hours and then returned to storage at 68°F.

To prepare the inocula for a given experiment, the stock cultures were transferred to Bacto nutrient agar slants and incubated 24 hours at 95°F. Following incubation, the inocula were prepared as reported earlier (Angelotti, *et al.*, 1959).

Preparation of Foods

Custard, ham salad, and chicken à la king were selected as foods representative of the types often incriminated in cases of food poisoning. They were prepared, distributed in 2-oz.

jars, and sterilized according to the procedure outlined formerly (Angelotti, *et al.*, 1959). Following sterilization and cooling, each screw-capped jar was placed in a separate vinyl plastic bag and suspended in a water bath in such a manner that the jar of food was completely submerged beneath the surface of the water. The end of the plastic bag was twisted and clamped in place along the edge of the water bath. This procedure prevented seepage of water into the jars of food, but insured that the contents of the jars were maintained at the bath temperature. The water baths were adjusted according to a Bureau of Standards thermometer and maintained the following temperatures (plus or minus 0.18°F): 95°F, 112°F, 114°F, 116°F, 118°F, and 120°F.

Inoculation and Bacteriological Examination

In each experiment, the responses of salmonellae or staphylococci were determined for a given food at four temperatures simultaneously. One-tenth-ml. aliquots of the pooled suspension of test organisms were inoculated into replicate jars of the food which had been brought to bath temperature as described above. This volume of suspension, added to the 50 g. of food in each jar, was an inoculum of approximately 10,000,000 cells per gram. After inoculation, the screw caps were replaced and the jars shaken vigorously by hand for 50 return strokes to distribute the inoculum throughout the food mass. Then they were returned to the plastic bags and once again suspended in the water baths until removed for bacteriological examination.

At 6-hour intervals for 24 hours, duplicate jars of food were removed from each water bath and the contents transferred into a sterile Waring Blendor cup to which 430 ml. of sterile, cold (45°F) phosphate dilution water (Am. Public Health Assoc., 1953) had been added. Each jar was rinsed with two additional 10-ml. aliquots of the dilution water and these were also added to the blender cup. The mixture was

blended two minutes at slow speed and appropriate 10-fold dilutions prepared from the blends in sterile phosphate dilution water. Duplicate plates of each dilution were poured, employing Bacto plate count agar, and the plates were incubated at 95°F for 24 hours.

To determine quantitatively the number of organisms per gram in the food immediately after inoculation, two additional jars of food were inoculated as described. These jars of food were at room temperature at the time of inoculation, and immediately after shaking the contents were blended, plated out, and incubated as described above.

One uninoculated jar of food also was incubated in each of the water baths to serve as a "leak" control. At the end of the 24-hour series, these jars were sampled and plated similarly to the test jars.

RESULTS

The growth responses of the salmonellae and staphylococci in the different foods at temperatures of 95°F through 120°F are shown in Figures 1 through 3 and in Tables 1 through 3 of Appendix A. In ham salad (Fig. 1 and Table 1) both groups of organisms grew well at 95°F; however, a decrease in numbers of organisms proportionate to temperature occurred at all temperatures from 112°F to 120°F.

In chicken à la king (Fig. 2 and Table 2) both the staphylococci and salmonellae displayed excellent growth at 95°F; however, notable differences were observed between the responses of the two groups at the remaining temperatures. The staphylococci grew well at 112°F, but died off at the higher temperatures. At 114°F, approximately 90% reduction of viable organisms occurred after 12 hours; whereas a similar reduction occurred at 116°F after 6 hours and at 118°F and 120°F in less than six hours. The salmonellae grew at 112°F and 114°F, but underwent a gradual reduction to 60% of the original inoculum after 24 hours at 116°F and to 50% at 118°F. At 120°F, the survivors after 24 hours represented 20% of the original inoculum.

In custard (Fig. 3 and Table 3), both groups again displayed excellent growth at 95°F. The staphylococci grew well at 112°F, showed

moderate growth at 114°F, and at 116°F underwent a slight increase during the first 12 hours and then a gradual decline between 18 and 24 hours. At 118°F and 120°F, a 90% reduction was observed after 24 and 6 hours, respectively. The salmonellae grew well at 112°F, displayed poor growth at 114°F, and at 116°F increased slightly during the first 6 hours, followed by a gradual decline between 18 and 24 hours. At 118°F, essentially no change in numbers was observed throughout the 24-hour period, whereas a gradual decline was observed resulting in a 60% reduction after 24 hours of incubation at 120°F.

In every instance, control jars of uninoculated food were negative for growth.

DISCUSSION

The results obtained in this study revealed that salmonellae and staphylococci are capable of growing in certain foods at temperatures as high as 112°F to 114°F; whereas at 116°F death occurs or the population density remains stable during 24 hours of storage. Previous studies, in which the growth response of these organisms was measured in the same foods held at refrigeration temperature, showed that growth could occur at 44°F and above (Angelotti, et al., 1959).

The general distribution of these two groups of organisms enables them to gain entry into foods easily from both human and animal sources. The common occurrence of staphylococci on the skin and in the naso-pharynx of man contributes to their frequent isolation from non-sterile foods. The prevalence of salmonellae in certain meat and poultry products provides an entry for these organisms into dishes prepared from them. Since these bacteria are vegetative organisms, neither group displays the heat resistance associated with bacterial endospores; consequently, they may be eliminated from foods which are pasteurized or cooked at temperatures that do not result in sterilization. However, after cooking and during the cooling process, the types of foods associated with staphylococcal and salmonella food poisoning often require handling. Boning chicken or mincing ham for salads, filling puffs, eclairs, etc., are examples of this type of handling. Thus, foods which have been freed of these organisms once again become contaminated. Though the level of con-

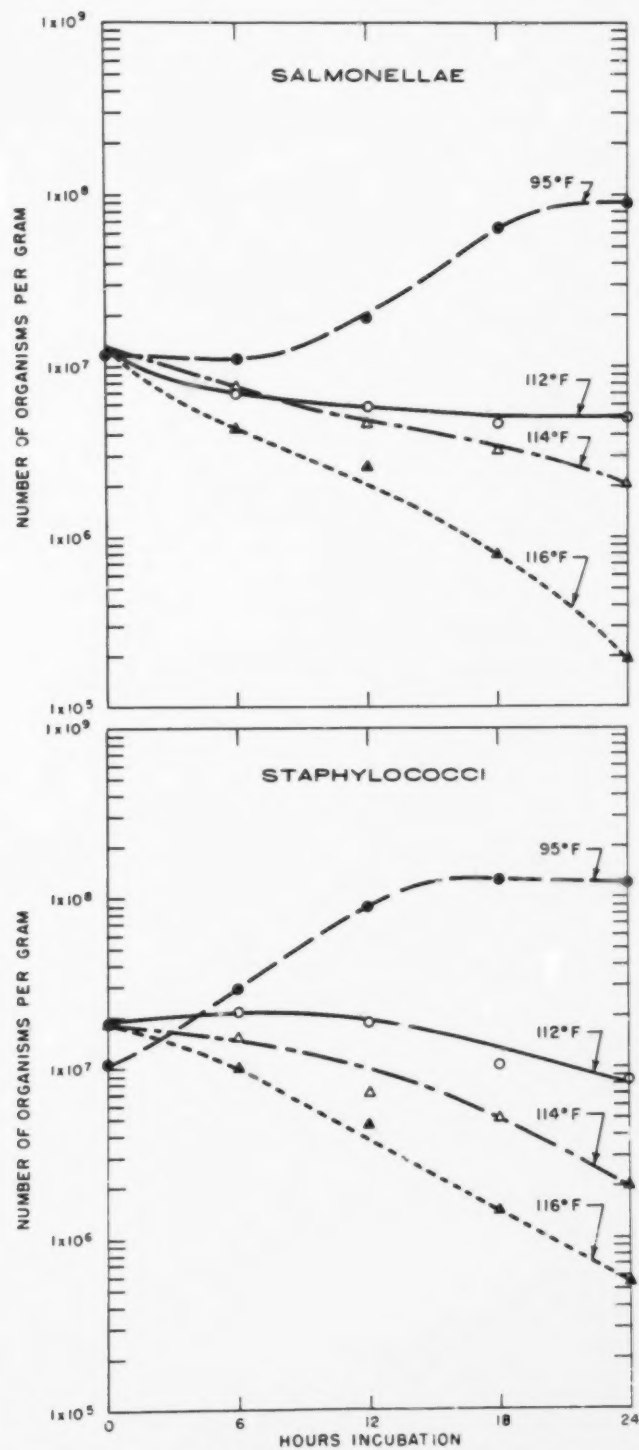


Figure 1. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN HAM SALAD INCUBATED AT VARIOUS TEMPERATURES.

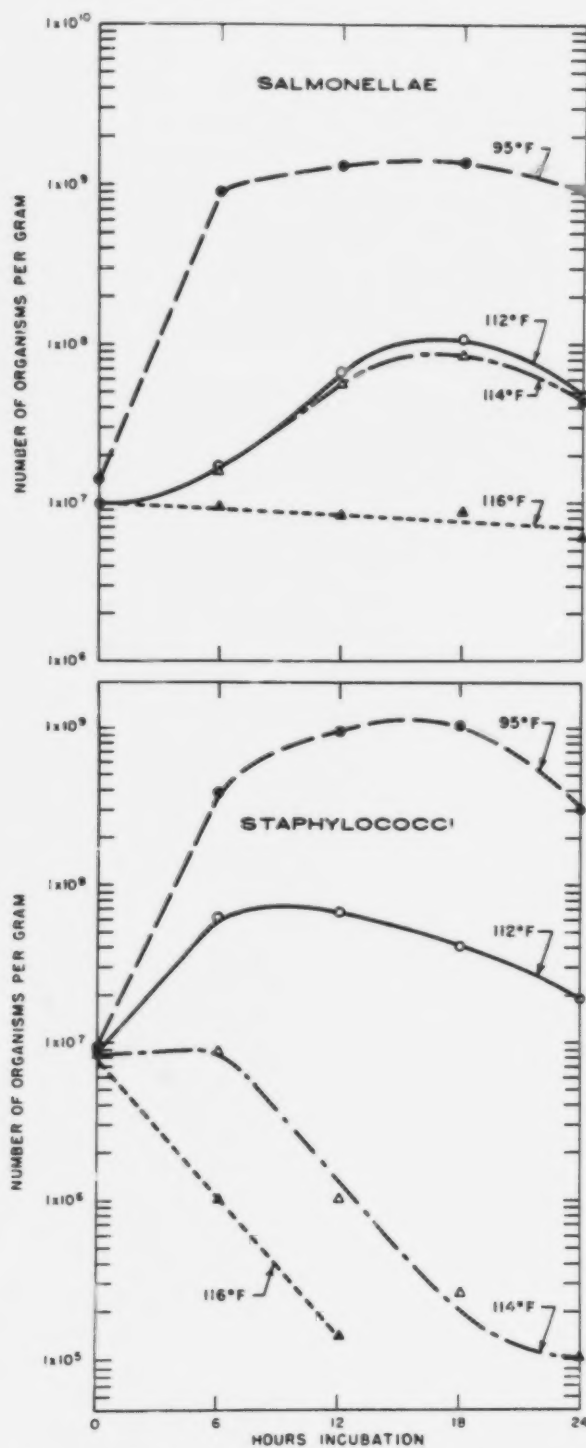


Figure 2. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN CHICKEN À LA KING INCUBATED AT VARIOUS TEMPERATURES.

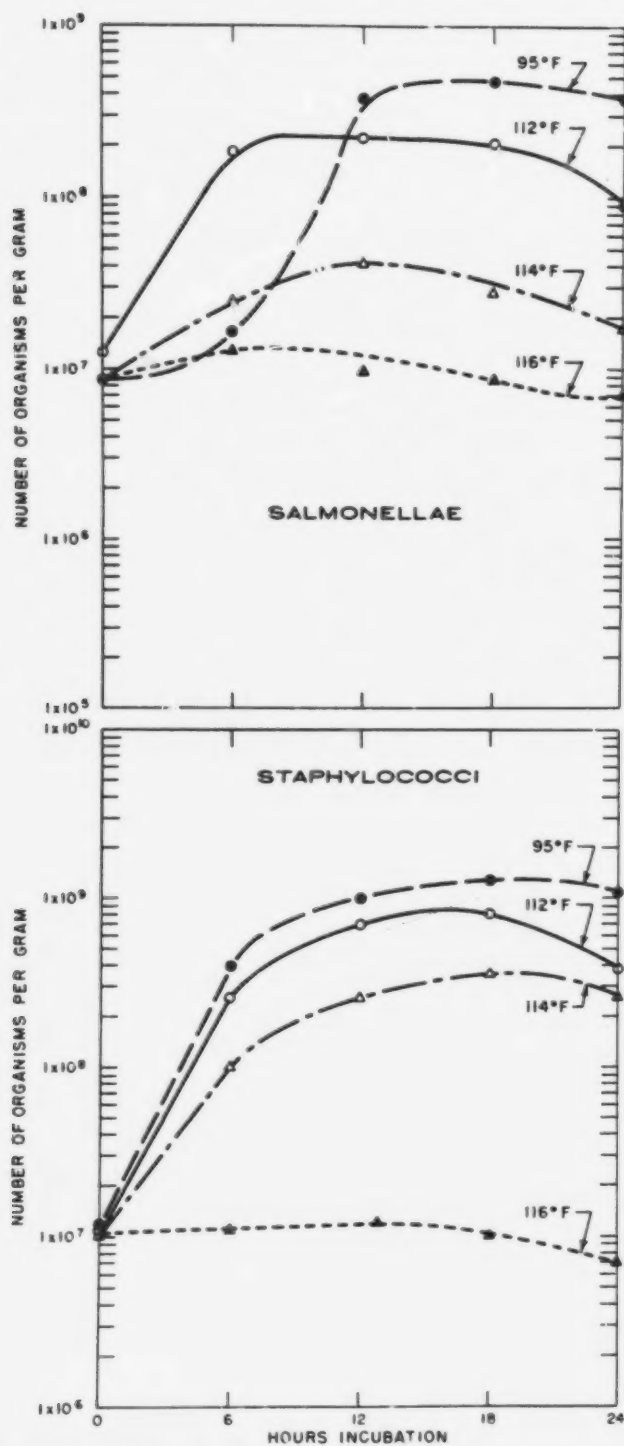


Figure 3. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN CUSTARD INCUBATED AT VARIOUS TEMPERATURES.

tamination at this point is usually insufficient to produce gastro-enteritis, the necessary level is rapidly attained when the conditions of time and temperature required for growth or toxin production are permitted to develop. It appears from the data reported here and that presented earlier (Angelotti, et al., 1959) that salmonellae and staphylococci will multiply in the range of 44°F to 114°F, and that extensive growth can occur in a few hours at the warmer temperatures.

Observations were not made on the effect of changing environmental temperature with time, as is done in "heat-up" and "cool-down" studies. However, with the knowledge of the relatively short generation time of bacteria under ideal conditions, and from the data presented by others (White and Longree, 1957; Longree and White, 1955; Dack, 1956) on cooling rates of large quantities of food, and of the growth of food-poisoning bacteria in such foods, maintaining the internal temperature of foods within the mid-range of the "incubation danger zone" for 6 hours or more must be regarded as a dangerous practice.

SUMMARY

Mixed cultures of S. senftenberg 775W, S. enteritidis, S. manhattan, and Staph. aureus

196E, Staph. aureus Ms149, and Staph. aureus MF159 were cultured in custard, chicken à la king, and ham salad for 24 hours at 2°F intervals from 112°F to 120°F and at 95°F.

In custard, both groups of organisms grew at 114°F, but decreased in numbers at 116°F through 120°F.

In chicken à la king, the staphylococci grew at 112°F and were gradually destroyed at all the higher temperatures. The salmonellae grew at 114°F but declined at 116°F and above.

In ham salad, both groups of organisms decreased in numbers at temperatures of 112°F through 120°F.

Good growth of the test organisms in all three foods was observed at 95°F.

The results indicate that the temperature growth range for salmonellae and staphylococci in foods of the type studied is 44°F to 114°F. The prevalence of these organisms in the environment provides so many opportunities for contamination of food that the risk is very great of their growing in perishable products exposed to the mid-portion of the growth-temperature-range for a few hours.

Part Two.- THERMAL DEATH TIME STUDIES ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

PURPOSE

Presently, considerable discussion is directed toward the temperature at which hot foods must be held in order to eliminate the danger of foodborne infection or intoxication. The Public Health Service recommends that readily perishable foods held within vending machines be maintained at a temperature not lower than 150°F (The Vending of Foods and Beverages, A Sanitation Ordinance and Code, PHS Publication No. 546); whereas 140°F is the recommended temperature for storing readily perishable foods aboard aircraft (Handbook on Sanitation of Airlines, PHS Publication No. 308). In the Ordinance and Code Regulating Eating and Drinking Establishments (PHS Publication No. 37) no recommendation is given concerning the temperature at which hot foods are to be held during preparation or service. The latter publication is now undergoing revision, and it is hoped that the results of this study may serve as a guideline for the development of recommendations on warm holding temperatures.

In view of conflicting recommendations and the trend in certain segments of the food service industries to lower the holding temperature to less than 150°F, and in some instances to below 140°F, these studies were undertaken to determine temperatures at which highly perishable foods may be held safely under steamtable or vending machine storage conditions. The studies were specifically designed to obtain data on the thermal resistance of food-poisoning salmonellae and enterotoxigenic staphylococci in foods held at temperatures of 130°F through 150°F.

METHODS

Preparation of Cultures

Previous research, conducted in our laboratory, on the heat tolerance of a number of

salmonellae species representative of those associated with foodborne salmonellosis and on several enterotoxigenic strains of Staphylococcus aureus permitted us to select the following organisms as those displaying the limits of heat tolerance typical of salmonellae and staphylococci (Angelotti, et al., 1959): high heat resistance, Salmonella senftenberg 775W, and Staphylococcus aureus Ms 149; low heat resistance, S. manhattan, and Staph. aureus 196E.

Initially all the organisms were grown on nutrient agar slants incubated at 95°F for 24 hours. They were harvested in sterile phosphate buffered dilution water (Am. Public Health Assoc., 1953), inoculated into sterile skim milk and lyophilized. The lyophilized cultures were maintained as stocks at 41°F.

To prepare a primary culture, a lyophilized stock was opened, transferred to a tube of Bacto brain heart infusion broth, and incubated 18 hours at 95°F. A transfer was made from the broth to a Bacto nutrient agar slant (primary culture) which was incubated for an additional 24 hours at 95°F. This primary culture was stored at 68°F and served as a source of inoculum for secondary cultures for 5 consecutive days. At the end of 5 days, the primary culture was discarded, a fresh lyophilized stock opened, and the cycle repeated to obtain a new primary culture.

Secondary cultures were prepared by inoculating a Bacto nutrient agar Roux bottle with cells from the primary culture and incubating at 95°F for 24 hours. After incubation, the cells were washed from the surface with sterile phosphate-buffered dilution water, shaken thoroughly with beads to break up clumps and adjusted to the desired concentration with a Coleman Junior Spectrophotometer at 620 millimicrons.

In every experiment, the cells employed to inoculate the test foods were obtained from a 24-hour-old secondary culture which had under-

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

gone only one transfer. This technique was used to avoid variation and inadvertant selection of types whose heat resistance differed from that of the parent strain.

Preparation of Sterile Foods

Custard, ham salad, and chicken à la king were prepared as described previously, except that the water was omitted from the ham salad and chicken à la king (Angelotti, *et al.*, 1959). The foods were weighed out in 250-g. aliquots and placed in 1-lb., screw-cap jars with rubber seals. The jars of ham salad and chicken à la king were autoclaved at 252°F for 45 minutes and the custard at 239°F for 45 minutes. Each batch of food prepared throughout the study was tested for sterility by removing the contents of a few jars and plating in Bacto plate count agar and inoculating into Bacto fluid thioglycolate medium. The results of these tests indicated that in every instance a sterile product was obtained. The sterile jars of food were stored at 41°F.

For each experiment, a single jar of food was employed. One ml. of the appropriately adjusted culture described above was inoculated into the cold jar of food and resulted in a cellular concentration of approximately 1×10^7 organisms per gram as determined by plate count. (See page 13, col. 2.) The inoculum was thoroughly mixed into the food with a sterile tongue depressor. Immediately after inoculation, the jar of food was placed in a 37°F water bath to prevent multiplication of the organisms during the interval required for setting up the thermal death time (TDT) tube filling apparatus. This interval was never more than 10 minutes.

Procedure for Filling, Holding, and Heat Treating TDT Tubes

In view of the success reported by Stumbo, *et al.* (1945), in filling TDT tubes with solid

foods, an "Allstate" (a)* spring-fed grease gun was used to fill the TDT tubes. The grease fitting was replaced with a length of borosilicate glass tubing (7 mm. x 120 mm.) held in place with a short section of rubber tubing. This attachment permitted the injection of the food into the bottom of the TDT tube in a continuous column free of entrapped air bubbles. Each tube received that amount of food delivered by one stroke of the gun. The average variation in weight delivered upon replicate strokes of the gun was found to be plus or minus 0.1 g. for all three foods. The mean weight delivered for each food differed slightly as follows: chicken à la king 1.1 g.; custard 1.0 g.; and ham salad 1.1 g.

All parts of the grease gun were thoroughly coated with a light covering of nontoxic, high melting point, technical grade, white grease (b) to prevent rusting during autoclaving. The head and barrel assemblies were wrapped separately in craft paper and autoclaved at 252°F for 30 minutes. After autoclaving, the assemblies were oven dried at approximately 195°F and refrigerated to 41°F.

To fill the TDT tubes, the sterile, cold gun was reassembled, filled aseptically with the cold inoculated food, and clamped to a ring stand. Soft glass, 100 mm. x 10 mm. TDT tubes were employed. For each thermal resistance test performed, 72 sterile, cotton-plugged tubes were distributed into 6 copper serological test tube racks containing 12 tubes per rack. As each rack of tubes was filled, the rack was placed in a 37°F (+ 0.18°F) water bath to prevent multiplication of the test organisms. After filling all 72 tubes, one tube at a time was removed from the 37°F bath, sealed in an oxy-gas flame, and returned to the bath. After sealing, the ends were permitted to cool sufficiently to prevent cracking and the TDT tubes were completely submerged in the 37°F bath to equilibrate for 10 minutes. The complete filling, sealing, and cooling operation required approx-

(a) Allstate, steel, spring-fed grease gun manufactured by K-P Manufacturing Co., Minneapolis, Minnesota and purchased from Sears Roebuck and Company.

(b) Petrol-Gel (white), McGlaughlin Oil Company, 3750 E. Livingston Ave., Columbus 13, Ohio.

* NOTE: Mention of commercial products is not to be construed as endorsement by the Public Health Service.

imately 60 to 90 minutes. Multiplication of the test organisms was prevented during this time by keeping the temperature of the food continuously below 45°F. Following equilibration to 37°F, all 6 racks of tubes were removed from the cold bath and plunged simultaneously into a water bath heated to the desired test temperature. The hot bath maintained the test temperature within + 0.18°F. Timing was begun at the instant of submersion and at selected intervals individual racks were withdrawn from the bath, plunged instantaneously into the 37°F bath, and cooled for 10 minutes.

In order to locate the limits of survival and determine the proper exposure times, preliminary runs were made with each organism in the various foods at 5°F intervals from 120°F to 150°F. For each test exposure, 10 TDT tubes were examined for survivors. The two extra tubes per time interval were used as replacements to fill out a 10-tube series in those instances where breakage occurred. The heat-treated TDT tubes were examined for survivors by two methods. In the case of chicken à la king and custard, the cooled tubes were incubated for 5 days at 95°F, after which they were scored with a motor-driven glass tubing cutter, snapped open, and a loopful of the contents transferred to a tube of Bacto phenol red broth containing 1% dextrose. The inoculated broth tubes were incubated at 95°F for 24 hours and examined for turbidity and acid production. TDT tubes containing ham salad were handled similarly to the others, except that they were opened and the contents transferred immediately after cooling 10 minutes. These two methods, out of several variations tried, resulted in the largest number of tubes showing survivors. In ham salad a 5-day incubation resulted in fewer survivors than the method finally adopted. It was also found necessary to employ a broth with an acid-base indicator, because the loopful of food added to the broth resulted initially in some turbidity.

All tubes showing survivors were examined by Gram stain. The salmonellae survivors were streaked onto Bacto brilliant green agar plates and incubated 48 hours at 95°F. Following incubation, colonies were picked and tested against *Salmonella* polyvalent serum. Surviving staphylococci were streaked onto Bacto staphylococcus 110 medium and incubated 48 hours at 95°F. After incubation, colonies were picked and tested for coagulase production.

To determine the number of organisms per gram in the inoculated foods, four additional TDT tubes were filled, sealed, and handled in a manner identical to the TDT tubes of the test series. Following the 10-minute cooling period, the control tubes were opened, their contents combined, and two 1.0 gram aliquots weighed out. Appropriate 10-fold dilutions of the aliquots were prepared in sterile, phosphate buffered dilution water and duplicate plate counts of each made in Bacto plate count agar. The plates were incubated at 95°F for 48 hours. In all cases the food was found to contain between 9×10^6 to 11×10^6 cells per gram.

Determination of Rate of Heat Penetration

TDT tubes were filled with one of the test foods as described above. An iron-constantan thermocouple (B & S 32-gauge) was placed in a tube with the hot junction at the geometric center of the food column. The thermocouple was centered in the tube by means of 3 plastic rods which held it firmly, and the tube was sealed with a water-tight rubber cap, through which the leads extended. The tube was submerged in the 37°F water bath, and the temperature was recorded every 7.2 seconds by means of a Leeds and Northrup Speedomax Type G temperature recorder. When the internal temperature reached 37°F, the tube was transferred to the hot water test bath and the interval required to raise its temperature to that of the test bath, as registered by a control thermocouple was recorded. On attaining this temperature, the tube was returned to the 37°F bath and the interval required to lower the internal temperature to 113°F was recorded. This procedure was repeated with five tubes of each food at 5°F intervals from 120°F to 150°F. The average temperature per time interval was calculated for each bath temperature employed. The temperature of 113°F was selected as an end point, because previous work in our laboratory revealed that thermal inactivation of the test organisms does not occur at this temperature or below. (Angelotti, *et al.*, Part I, this report.) Using the average values, heat penetration curves were drawn for the three foods for each bath temperature (Ball, 1943; Schultz and Olson, 1940; Anellis, *et al.*, 1954) as follows: semi-log paper is rotated 180° and time is plotted on the abscissa against number of degrees below bath temperature and internal food temperature on the logarithmic coordinates with 0.1°F below

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on the logarithmic coordinates with 0.1°F below bath temperature as the endpoint. This type of curve is shown in Figure 1.

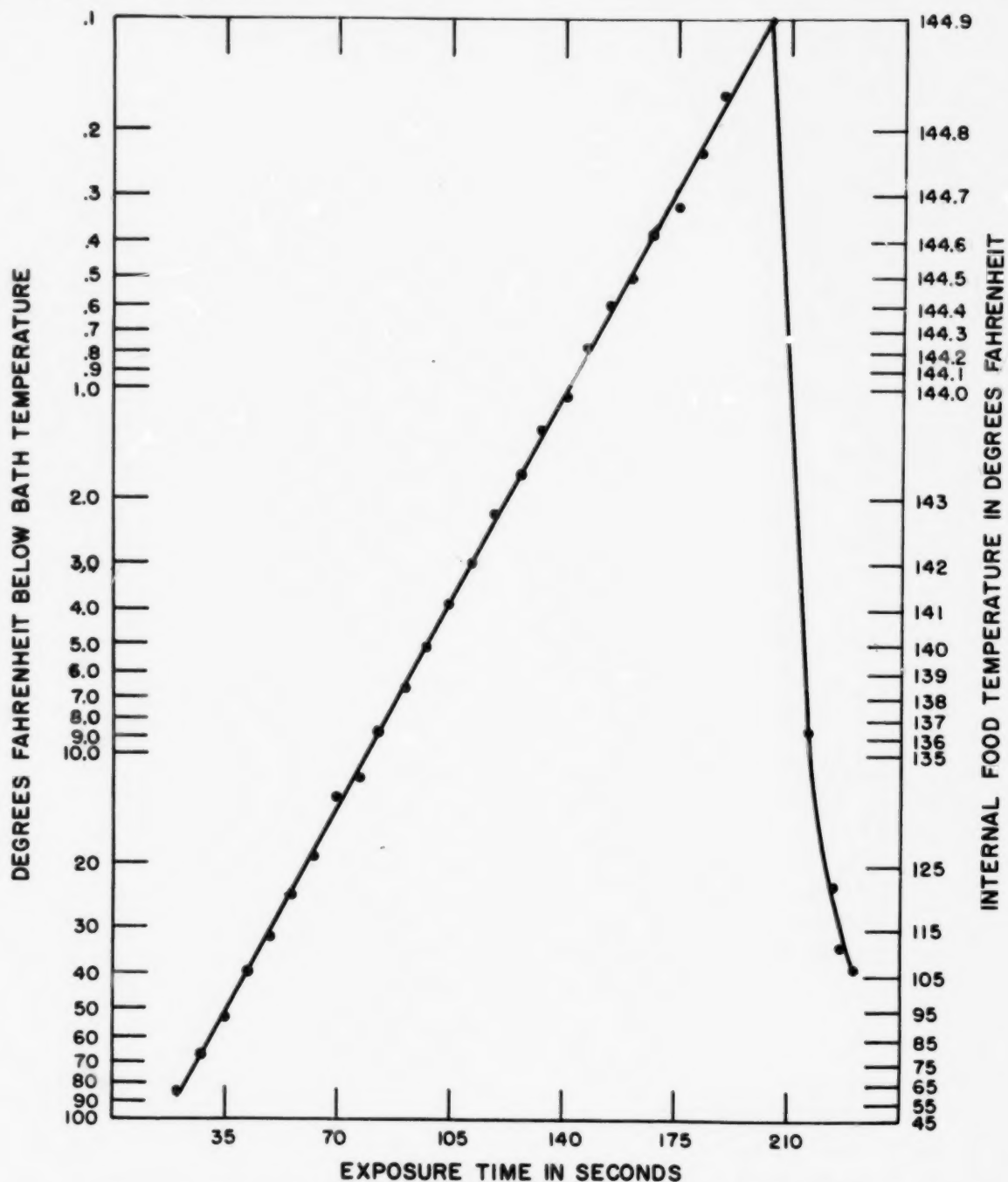


Figure 1. RATE OF HEAT PENETRATION INTO 1.0 GRAM OF CUSTARD CONTAINED IN 100 x 10 mm. SOFT GLASS TUBES. AVERAGE OF 5 REPLICATE DETERMINATIONS FOR BATH TEMPERATURE 145°F .

bath temperature as the endpoint (Figure 1).

In calculating thermal death times, correction is necessary for "thermal lag," (the interval required for the slowest heating portion of the food to reach bath temperature, followed by cooling to a non-lethal temperature), as well as correction for "lethality during lag" (the degree of destruction which occurs during the time required to reach bath temperature, plus that occurring during the cooling period). These corrections were made as follows: uncorrected thermal-death-time curves were plotted in the conventional manner on semilog paper and straight lines drawn to best fit the points of "survival-kill." The lethal rate value associated with each point (Bigelow, *et al.*, 1920; Schultz and Olson, 1940; Ball, 1943; Anellis, *et al.*, 1954) on the heat penetration curves was calculated by taking the reciprocal of the time (number of minutes) required to kill on a given thermal-death-time curve and plotting this reciprocal against the time (number of seconds) required to reach this temperature on the corresponding heat penetration curve. This value is shown as: lethal rate = $1/\text{killing time}$. Enough of these values were obtained so that a smooth lethality curve resulted when the lethal rates were plotted on linear coordinates against the times required to reach these temperatures on the heat penetration curve. This type of curve (Figure 2) was drawn for each bath temperature and food employed and the area under each curve was measured with a planimeter. This area (A) represented the degree of bacterial destruction which occurred during the actual heating and cooling of the inoculated food. A rectangle (A') was drawn on the lethality curve coordinates with the ordinate equal to the highest lethal rate of a given bath temperature and the abscissa corresponding to one minute of heating. The area of this rectangle represented the lethality occurring during a heat treatment involving instantaneous heat-up, holding for one minute, and instantaneous cool-down. The ratio of the areas A/A' for each bath temperature, provided the calculated correction factor representing additional minutes of lethal exposure occurring during come-up and come-down. The formula for expressing heat exposure time corrected for thermal lag and lethality during lag (Anellis, *et al.*, 1954) is as follows:

Corrected Exposure Time = Total exposure time in bath — thermal lag time + lethality due to time of come-up and come-down

The differences between heat penetration times and lethalties yielded a mean correction factor for each of the foods as follows: custard, 1.6; chicken à la king, 1.5, and ham salad, 1.4. These values correspond very closely to those reported by others for various foods in glass TDT tubes (Ball, *et al.*, 1937; Sognefest and Benjamin, 1944; Anellis, *et al.*, 1954; Osborne, *et al.*, 1954). These values, subtracted from the total exposure time in the bath, gave endpoints of "survival-kill" corrected for thermal lag and lethality due to lag. Employing these points, corrected thermal-death-time curves were constructed and defined in terms of their F_{140} (minutes exposure at 140°F necessary to reduce the inoculum to level which is not detectable) and z_F values (number of °F necessary for the thermal-death-time curve to traverse one log cycle). These corrections are insignificant when the heating times are long, but may be significant when the times are of relatively short duration. For this reason, a greater degree of certainty is associated with the survival-kill points of the corrected TDT curves at the longer exposure times than at those for exposures of less than 10 minutes.

D values (minutes' exposure at 140°F necessary to effect a 90% reduction in original inoculum) were calculated by the probability procedure of Schmidt (1957) and decimal reduction time curves were constructed.

RESULTS

The results obtained in custard and chicken à la king with the test salmonellae and staphylococci are summarized in Tables 1 and 2. The F_{140} , z_F , D_{140} , and z_D values obtained in duplicate experiments are shown along with average values. Figures 1 through 8 of Appendix B show the thermal death time and decimal reduction time curves obtained from plotting average values. It is to be emphasized here that TDT and D values determined in the manner described above represent endpoints of survival of the most heat-resistant cells present in a given population.

These data reveal several points of interest in respect to the thermal resistance of the two groups of organisms in chicken à la king and custard. As would be expected, rather large differences in the heat resistance of the salm-

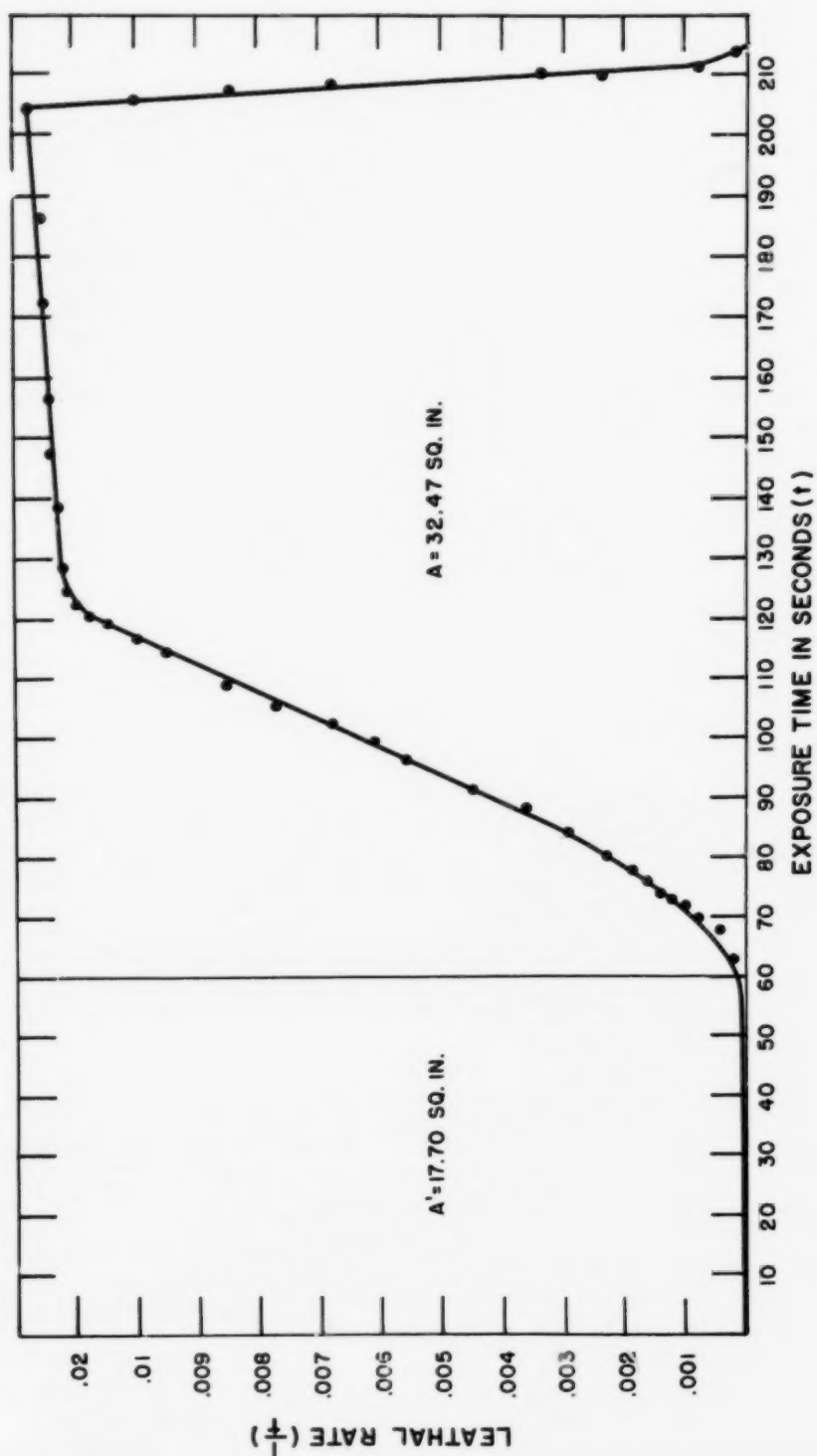


Figure 2. LETHALITY CURVE FOR *STAPH. AUREUS* Ms149 IN CUSTARD; APPROXIMATELY 1×10^7 CELLS PER TUBE.

onellae and staphylococci were observed. Excluding, momentarily, the results obtained with the exceptionally heat-resistant S. senftenberg 775W (Solowey, *et al.*, 1948; Anellis, *et al.*, 1954; Osborne, *et al.*, 1959; Angelotti, *et al.*, 1959), the staphylococci displayed greater heat resistance than the remaining salmonella species. This is in keeping with the findings for broth cultures reported earlier (Angelotti, *et al.*, 1959). Also, in keeping with these findings, very little difference in heat resistance was observed among the staphylococci; however, Staph. aureus 196E gave greater F_{140} values than Ms149. This was unexpected, in view of the earlier work which indicated that broth cultures of Ms149 were more heat resistant than 196E.

S. senftenberg 775W displayed the greatest heat resistance of any of the organisms studied. The F_{140} values in custard (78.0) and chicken à la king (81.5) were quite similar. It is important to note, however, that the extreme heat tolerance of S. senftenberg 775W is not a common property of this serological type as was shown by Anellis, *et al.* (1954), with strains 523, 2623, and 3252 which were also isolated from egg products. It is equally important to note that S. senftenberg 775W was originally isolated from egg products; and, therefore, it is reasonable to assume it may, on occasion, contaminate prepared foods containing eggs. The z values of 11.45 in chicken à la king and 11.8 in custard are comparatively greater than the values observed for the other organisms. Though greater, they still fall within the generally accepted value for z of 10 ± 2 for vegetative organisms in the range of 130° to 160°F (Schmidt, 1957). The higher z value shown for this organism emphasizes the need for a high holding temperature for foods in which it is apt to occur.

S. manhattan was the least heat-resistant organism studied and yielded F_{140} values more in keeping with those generally recognized for salmonellae (Solowey, *et al.*, 1948; Anellis, *et al.*, 1954; Osborne, *et al.*, 1954). Custard afforded greater protection against heat destruction than chicken à la king, and the slope of the TDT curve in custard was not as steep. This was the only instance observed in which the food menstruum apparently affected the slope of the TDT curve. Kaplan, *et al.* (1954), has shown that z values are independent of the menstruum in which the organism is heated, and that the

observed variation in individual z values for different foods fall within statistical limits of experimental error. The variation for z observed with S. manhattan in custard and chicken à la king may represent such experimental error in that both are low acid foods and neither contain any substances in great enough concentration to inhibit survivors upon product incubation at 95°F (Angelotti, *et al.*, 1959).

In custard and chicken à la king, both strains of Staph. aureus were found to possess an intermediate degree of heat resistance between the high of S. senftenberg 775W and the low of S. manhattan (Tables 1 and 2 and Figures 1 through 8, Appendix B).

Under practical conditions, ham salad would not be served as a hot food, however, a few trials were made with this food for comparative purposes. These experiments revealed that both the salmonellae and staphylococci gave lower end points of survival than in custard or chicken à la king. Because holding temperatures for hot foods are best based on the survival of the most heat-resistant organism likely to be encountered in a food, affording it maximum heat protection, little merit was associated with determining TDT and D values for the test organisms in ham salad. However, Table 3 shows the "survival-kill" points attained with S. senftenberg 775W and Staph. aureus Ms149 in this food.

For comparative purposes, D_{140} values are also presented in Tables 1 and 2. These data show the relative heat resistances of the organisms to be similar to that shown by the F_{140} data. The D_{140} data emphasize the relatively short period of time, from that of the whole heat treatment, required to kill 90% of the bacterial population. Because D is independent of initial bacterial concentration (Youland and Stumbo, 1953; Pilug and Esselen, 1954; Schmidt, 1957), although some evidence exists to the contrary (Reed, *et al.*, 1951; El-Bisi and Ordal, 1956^a, 1956^b) a greater flexibility is available for interpreting heat resistance data than is possible by thermal-death-time determinations alone. When D values are plotted on a logarithmic scale versus temperature on a linear scale (See Figures 1 through 8 of Appendix B.) a straight line is obtained for the relationship between log D and temperature (Stumbo, *et al.*, 1950; Reynolds and Lichtenstein, 1952). This represents the "phantom" thermal-death-time

curve of Ball (1943). However, this curve can be characterized with a point and a slope by substituting D and z_D for F and z_F of the thermal-death-time curve, which gives it both direction and position, as opposed to Ball's "phantom" curve, which had neither direction nor position. Schmidt (1957) points out that the D curve represents the real heat resistance of the organism and because it is based on assumptions of a logarithmic order of death, is valid in those instances where a logarithmic rate of destruction occurs. From the time of Bigelow and Esty (1920), it has been recognized that the heat resistance of an organism is dependent upon initial concentration. Because of this, F values for different suspensions are not comparable, unless the initial inocula were identical. By employing D , it is possible to determine the heat resistance of an organism and make direct comparisons between data in which various sized inocula were employed. In freeing the investigator from dependence upon inoculum size, a method is available that enables him to determine thermal resistance in a given substrate under every conceivable condition of bacterial load. This fact is particularly important in a study of the type presented here. From a practical standpoint, it is impossible to measure the heat resistance of an organism in prepared dishes under every possible condition of bacterial load. But in calculating D values, a measure of thermal resistance is derived, based on a limited number of experiments, which is valid through the whole range of predictable bacterial concentrations attainable in a given food.

DISCUSSION

Judging from the values presented for D_{140} , 90% destruction of the population of the most heat-resistant organism studied (*S. senftenberg* 775W) occurred in approximately 10 to 11 minutes in both custard and chicken à la king, and the same degree of destruction for the staphylococci occurred in approximately 5 to 8 minutes. When the number of salmonellae remaining in a food after a 90% reduction is not equivalent to an infective dose, little danger is associated with the ingestion of such a food. According to McCullough and Eisele (1951), the infective dose for several strains of *S. melagris* and *S. anatum* ranged between 587 thousand to 67.2 million. However, in those instances where a 90% reduction does result in numbers

approximating an infective dose, danger still exists. With *S. senftenberg* 775W in chicken à la king, all the cells were killed after 81.5 minutes' exposure at 140°F and a 90% reduction occurred after 9.61 minutes. From the slope of thermal-death-time curve (z_F 11.45) and the decimal-reduction-time curve (z_D 11.83), we find that a temperature of 152°F would yield the same results, but with better than a ten-fold reduction in time. However, even this information does not tell the investigator whether an infective dose exists after such a heat treatment, unless he knows the initial concentration of cells; a situation rarely encountered in practice. But, by employing D alone, any arbitrary degree of destruction can be achieved irrespective of the initial concentration. For example, if $D = 90\%$ reduction, then a process of $2D = 99\%$ reduction, etc. Assuming an exaggerated situation in which 1×10^7 salmonellae per gram of food are present, a process equivalent to $6D$ would yield a 99.9999% reduction in numbers, or 10 salmonellae per gram. A 100-gram serving would contain 1,000 organisms. From the data presented, it is seen then that a process of $6D$ would have reduced ten million *S. senftenberg* 775W per gram of custard (the food providing it greatest protection) to an innocuous level of 10 salmonellae per gram, after 67.92 minutes' exposure at 140°F. At the same time, this exposure results in complete destruction in either food of *S. manhattan* and the staphylococci. However, an extended exposure of this type may not be practical. From observing the decimal reduction time curve, it is seen that 90% reduction of *S. senftenberg* 775W in custard occurs after 1.68 minutes at 150°F. A process of $6D_{150}$ (10.08 minutes at 150°F) results in the same degree of destruction of *S. senftenberg* 775W in custard, as cited in the above example, and simultaneously gives complete destruction of this organism in chicken à la king, as well as complete destruction of the remaining organisms in all the foods.

Because z is independent of the menstrum in which vegetative organisms are heated, and because D provides a measurement of heat resistance independent of initial concentration of cells, a method is available of evaluating holding times and temperatures necessary to eliminate salmonellae and staphylococci from foods. The extreme heat resistance of *S. senftenberg* 775W and its occurrence in egg products demands that any heat treatments proposed to

Thermal Death Time Studies

Table 1. - HEAT RESISTANCE OF SALMONELLAE^(a) IN CUSTARD AND CHICKEN À LA KING

Organism	Custard				Chicken à la king			
	F ₁₄₀	z _F	D ₁₄₀	z _D	F ₁₄₀	z _F	D ₁₄₀	z _D
<u>S. senftenberg</u> 775W	78.00	11.80	9.36	12.25	83.00	11.00	9.22	12.15
<u>S. senftenberg</u> 775W	78.00	11.80	13.27	11.86	80.00	11.90	9.99	11.50
Average	78.00	11.80	11.32	12.05	81.50	11.45	9.61	11.83
<u>S. manhattan</u>	19.00	13.75	2.39	15.80	3.00	9.50	0.40	9.20
<u>S. manhattan</u>	19.00	13.75	2.49	13.00	3.10	9.10	0.39	8.70
Average	19.00	13.75	2.44	14.40	3.00	9.30	0.40	8.95

(a) Approximately 10,000,000 organisms per tube.

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 2. - HEAT RESISTANCE OF STAPHYLOCOCCI^(a) IN CUSTARD AND CHICKEN À LA KING

Organism	Custard				Chicken à la king			
	F ₁₄₀	z _F	D ₁₄₀	z _D	F ₁₄₀	z _F	D ₁₄₀	z _D
<u>S. aureus</u> 196E	59.00	10.50	8.24	9.90	47.00	10.50	5.25	9.95
<u>S. aureus</u> 196E	59.00	10.50	7.40	10.90	47.00	10.50	5.49	9.50
<u>Average</u>	59.00	10.50	7.82	10.40	47.00	10.50	5.37	9.73
<u>S. aureus</u> Ms149	52.00	9.20	7.68	9.20	38.00	9.40	4.89	8.95
<u>S. aureus</u> Ms149	54.00	10.70	7.68	9.85	42.00	9.20	5.45	10.50
<u>Average</u>	53.00	9.95	7.68	9.53	40.00	9.30	5.17	9.73

(a) Approximately 10,000,000 organisms per tube.

Thermal Death Time Studies

Table 3. - HEAT RESISTANCE OF SALMONELLA SENFTENBERG 775W AND STAPHYLOCOCCUS AUREUS Ms149 IN HAM SALAD(a)

Organism	Temperature of exposure	Minutes of exposure	
		Last interval at which some tubes were positive.	First interval at which all tubes were negative.
<u>S. senftenberg</u> 775W	135°F	78.0	83.0
	140°F	32.0	35.0
	150°F	4.5	5.5
<u>S. aureus</u> Ms149	130°F	185.0	200.0
	140°F	35.0	40.0
	150°F	5.0	6.0

(a) Approximately 10,000,000 organisms per tube.

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

eliminate salmonellae from foods be based on the requirements necessary to destroy this organism. Fortunately, the F_{140} value for other strains of salmonellae, as shown here and reported by others (Winter, *et al.*, 1946; Solowey, *et al.*, 1948; Goresline, *et al.*, 1951; Osborne, *et al.*, 1954; and Anellis, *et al.*, 1954) is of the order of 3 to 4. These values are considerably less than required for *S. senftenberg* 775W. Heat resistant, enterotoxigenic staphylococci also require shorter heating times (F_{140} of 40 to 59). Therefore, a process capable of destroying *S. senftenberg* 775W would necessarily destroy other salmonellae and staphylococci with some margin of safety.

Because the extended time necessary to kill at 140°F may be impractical under actual conditions of food service, higher holding temperatures may be advantageous. The results reported here indicate that heating perishable foods of the type employed in this study to 150°F and holding every particle of food at this temperature for no less than 12 minutes reduces 10 million or fewer salmonellae or staphylococci per gram to levels not detectable by our methods. In practice, exposure times of longer duration at 150°F or of equivalent duration at temperatures above 150°F would provide a safety factor.

Because destruction time increases with a decrease in temperature, prolonged holding at 140°F is necessary to achieve results comparable to those cited above. However, if the level of contamination were approximately 1,000 organisms per gram of the test food, it is estimated that a process equivalent to $4D_{140}$ or 45 minutes at 140°F would, in most instances, eliminate detectable survivors.

Though this study was limited in the number of foods and organisms employed, the information has wide application. The value z as originally defined by Ball is the number of degrees F for the thermal death time curve to pass over one log cycle. In actuality, it represents the temperature coefficient for the change in rate of destruction with temperature. This value is relatively constant and is $z = 18 \pm 2$ for spores in the range 220-270°F and $z = 10 \pm 2$ for vegetative cells in the range 130-160°F. This indicates that the coefficient for the change in rate is constant and that differences between organisms and conditions of heating are not sig-

nificant. Until such time that sufficient data are available for showing statistically that differences in z values are significant for different organisms and conditions, the concept of the constancy of z is very useful for it states that a 10°F increase or decrease in temperature will produce a ten-fold increase or decrease in destruction time for vegetative cells, irrespective of the food in which they are heated. Therefore, by determining D experimentally in a given food for a vegetative organism and assuming $z_D 10$, it is possible to select a time and temperature which results in any desired degree of destruction for vegetative pathogens associated with that food in concentrations that are encountered in practice.

SUMMARY

Thermal-death-time studies were conducted at 5°F intervals from 130°F to 150°F with strains of salmonellae and enterotoxigenic staphylococci. Heat-resistant *S. senftenberg* 775W and *Staph. aureus* 196E and non-heat resistant *S. manhattan* and *Staph. aureus* Ms149 were studied in custard, chicken à la king, and ham salad.

The F_{140} values (minutes of exposure at 140°F required to effect 100% destruction) were as follows:

S. senftenberg 775W, custard 78, and chicken à la king 81.5; *S. manhattan*, custard 19, and chicken à la king 3.1; *Staph. aureus* 196E, custard 59, and chicken à la king 47; *Staph. aureus* Ms 149, custard 53, and chicken à la king 40.

The end points of survival-kill at all the test temperatures for both salmonellae and staphylococci in ham salad were considerably less than for the other foods studied.

D_{140} values (minutes of exposure at 140°F required to effect a 90% reduction in numbers) were also calculated from the data and presented.

Values for z_F and z_D (slope of the thermal-death-time and decimal-reduction-time curves) are also presented and discussed in relation to type of food, organism and temperature.

These data indicate that heating perishable foods of the type studied to 150°F and holding

every particle of food at this temperature for at least 12 minutes reduces 10 million or less salmonellae or staphylococci per gram to non-detectable levels.

The same degree of destruction is achieved in similarly contaminated foods when held at

140°F for 78 to 83 minutes.

On the basis of the calculation procedures presented, it is estimated that 45 minutes' exposure at 140°F would be necessary to reduce 1,000 organisms per gram to non-detectable levels.

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APPENDIX A

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 1. - BEHAVIOR OF SALMONELLA AND STAPHYLOCOCCUS IN HAM SALAD INCUBATED AT VARIOUS TEMPERATURES

Incuba- tion Temper- ature	Sample No.	Number of organisms per gram x 10 ⁻⁶ after incubation for:							
		Salmonella				Staphylococcus			
		6 hrs.	12 hrs.	18 hrs.	24 hrs.	6 hrs.	12 hrs.	18 hrs.	24 hrs.
95°F	1	11.80	15.00	64.00	78.00	33.00	88.00	142.00	144.00
		13.10	16.70	66.00	66.00	44.00	99.00	137.00	128.00
	2	10.00	23.00	61.00	100.00	20.10	76.00	100.00	113.00
		10.00	23.60	74.00	109.00	21.30	98.00	135.00	110.00
112°F	1	7.10	6.40	4.00	5.30	21.50	20.10	9.70	12.20
		6.70	6.80	3.30	5.50	23.20	20.10	11.80	9.50
	2	7.50	4.90	5.20	4.80	25.70	19.70	9.30	7.00
		8.50	5.20	6.10	4.50	26.20	15.90	11.80	6.00
114°F	1	7.50	4.9	1.95	3.38	16.40	7.90	5.30	1.71
		7.30	6.8	1.64	2.89	17.10	7.90	4.70	1.84
	2	7.60	3.30	5.00	1.17	15.90	6.80	4.80	3.10
		8.90	3.40	4.30	1.30	11.40	9.10	6.50	3.40
116°F	1	3.50	2.14	1.26	.10	9.10	4.60	1.44	.54
		3.70	2.89	1.03	.09	10.40	5.60	1.29	.71
	2	4.60	3.00	.55	.09	11.30	3.80	1.71	.46
		5.40	2.91	.35	.04	9.60	4.40	1.34	.47
118°F	1	2.50	1.50	0.45	0.050	2.63	1.37	0.49	0.078
		2.35	1.30	0.43	0.035	2.84	1.75	0.35	0.088
	2	2.80	1.75	0.36	0.040	3.30	1.58	0.46	0.070
		2.68	1.68	0.41	0.035	3.34	1.68	0.47	0.080
120°F	1	1.25	0.50	0.04	0.015	1.13	0.316	0.049	0.016
		1.00	0.45	0.05	0.020	1.19	0.440	0.054	0.018
	2	1.32	0.36	0.02	0.010	1.48	0.440	0.063	0.014
		1.40	0.55	0.01	0.015	1.35	0.420	0.051	0.016

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella* senftenberg 775W, *S. enteritidis*, and *S. manhattan*, and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 196E, *Staph. aureus* Ms149, and *Staph. aureus* MF159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = 12-13 x 10⁶/gm.; staphylococci = 10-18 x 10⁶/gm.

(c) All uninoculated control jars of food were negative for growth.

Behavior of Incubation at Various Temperatures

Table 2. - BEHAVIOR OF SALMONELLA AND STAPHYLOCOCCUS IN CHICKEN À LA KING INCUBATED AT VARIOUS TEMPERATURES

Incuba- tion Temper- ature	Sample No.	Number of organisms per gram x 10 ⁻⁶ after incubation for:							
		Salmonella				Staphylococcus			
		6 hrs.	12 hrs.	18 hrs.	24 hrs.	6 hrs.	12 hrs.	18 hrs.	24 hrs.
95°F	1	1,320.00	1,170.00	1,160.00	910.00	480.00	870.00	1,090.00	370.00
		1,220.00	1,240.00	1,160.00	990.00	430.00	850.00	1,100.00	330.00
	2	1,090.00	960.00	1,180.00	1,100.00	290.00	1,040.00	1,760.00	260.00
		920.00	1,040.00	1,060.00	990.00	380.00	950.00	1,670.00	310.00
112°F	1	17.00	63.00	109.00	47.00	77.00	53.00	55.00	19.00
		17.40	60.00	108.00	48.00	69.00	74.00	39.00	20.40
	2	17.30	56.00	93.00	49.00	61.00	84.00	36.00	18.30
		15.90	79.00	91.00	45.00	43.00	66.00	40.00	18.60
114°F	1	18.40	55.0	84.00	46.00	10.00	1.13	.12	.18
		17.20	60.0	89.00	52.00	7.00	1.24	.17	.24
	2	17.60	59.00	86.00	45.00	9.50	1.33	.19	.15
		15.80	54.00	87.00	40.00	9.80	1.43	.22	.14
116°F	1	9.30	8.40	8.90	6.50	1.25	.15	<0.01	<0.001
		9.50	8.00	9.70	6.90	1.00	.12	<0.01	<0.001
	2	9.40	8.30	8.60	6.30	1.28	.19	<0.01	<0.001
		9.90	8.20	8.40	5.80	1.18	.11	<0.01	<0.001
118°F	1	9.90	8.00	7.30	5.00	0.90	0.01	<0.001	0.0
		9.85	8.85	7.50	4.50	0.85	0.03	<0.001	0.0
	2	10.00	7.95	6.80	6.00	0.88	0.02	<0.001	0.0
120°F	1	8.80	6.65	3.75	2.25	1.09	<.003	0.0	0.0
		8.90	6.72	3.80	2.00	0.90	<.005	0.0	0.0
	2	7.75	6.80	4.00	2.35	0.89	<.004	0.0	0.0
		8.95	6.65	3.35	2.50	1.00	<.005	0.0	0.0

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella* senftenberg 775W, *S. enteritidis*, and *S. manhattan*, and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 196E, *Staph. aureus* Ms149, and *Staph. aureus* MF159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = 10-15 x 10⁶/gm.; staphylococci = 8-10 x 10⁶/gm.

(c) All uninoculated control jars of food were negative for growth.

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 3. - BEHAVIOR OF SALMONELLA AND STAPHYLOCOCCUS IN CUSTARD INCUBATED AT VARIOUS TEMPERATURES

Incuba- tion Temper- ature	Sample No.	Number of organisms per gram x 10 ⁻⁶ after incubation for:							
		Salmonella				Staphylococcus			
		6 hrs.	12 hrs.	18 hrs.	24 hrs.	6 hrs.	12 hrs.	18 hrs.	24 hrs.
95°F	1	20.60	400.00	510.00	370.00	320.00	930.00	1,170.00	1,060.00
		11.00	390.00	370.00	310.00	430.00	1,030.00	1,290.00	1,140.00
	2	17.70	350.00	460.00	410.00	450.00	930.00	1,410.00	1,220.00
		17.20	350.00	510.00	430.00	440.00	1,170.00	1,370.00	1,260.00
112°F	1	146.00	202.00	218.00	85.00	234.00	660.00	950.00	440.00
		169.00	232.00	217.00	85.00	195.00	810.00	810.00	300.00
	2	249.00	226.00	198.00	84.00	330.00	780.00	600.00	470.00
		189.00	218.00	196.00	110.00	260.00	660.00	850.00	450.00
114°F	1	20.90	33.00	33.10	17.80	120.00	226.00	400.00	276.00
		19.40	45.00	32.00	20.50	88.00	258.00	360.00	232.00
	2	31.00	43.00	26.90	17.80	103.00	287.00	340.00	300.00
		28.60	49.00	19.60	16.10	99.00	265.00	340.00	302.00
116°F	1	10.00	9.00	9.10	9.00	11.10	13.70	12.10	8.80
		10.50	8.00	8.50	6.40	10.90	12.60	8.60	8.10
	2	10.00	10.10	9.00	6.40	12.10	9.40	8.50	6.20
		11.00	10.30	7.70	6.90	9.70	11.50	10.90	5.80
118°F	1	10.00	11.00	10.00	9.95	8.60	5.90	2.60	1.25
		9.90	10.50	11.00	10.00	7.50	6.55	2.75	1.30
	2	10.50	9.95	10.78	11.00	7.75	4.32	3.00	1.42
		10.45	9.90	10.45	10.75	6.45	3.85	2.45	1.30
120°F	1	10.50	8.00	6.75	4.45	1.52	0.45	< 0.001	0.0
		10.00	7.75	5.85	4.00	1.91	0.42	< 0.001	0.0
	2	9.95	8.85	7.00	3.95	1.71	0.37	< 0.001	0.0
		10.00	7.00	6.89	4.00	1.40	0.40	< 0.001	0.0

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella senftenberg* 775W, *S. enteritidis*, and *S. manhattan*, and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 196E, *Staph. aureus* MS149, and *Staph. aureus* MF159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = $9-13 \times 10^6$ /gm.; staphylococci = $9-12 \times 10^6$ /gm.

(c) All uninoculated control jars of food were negative for growth.

APPENDIX B

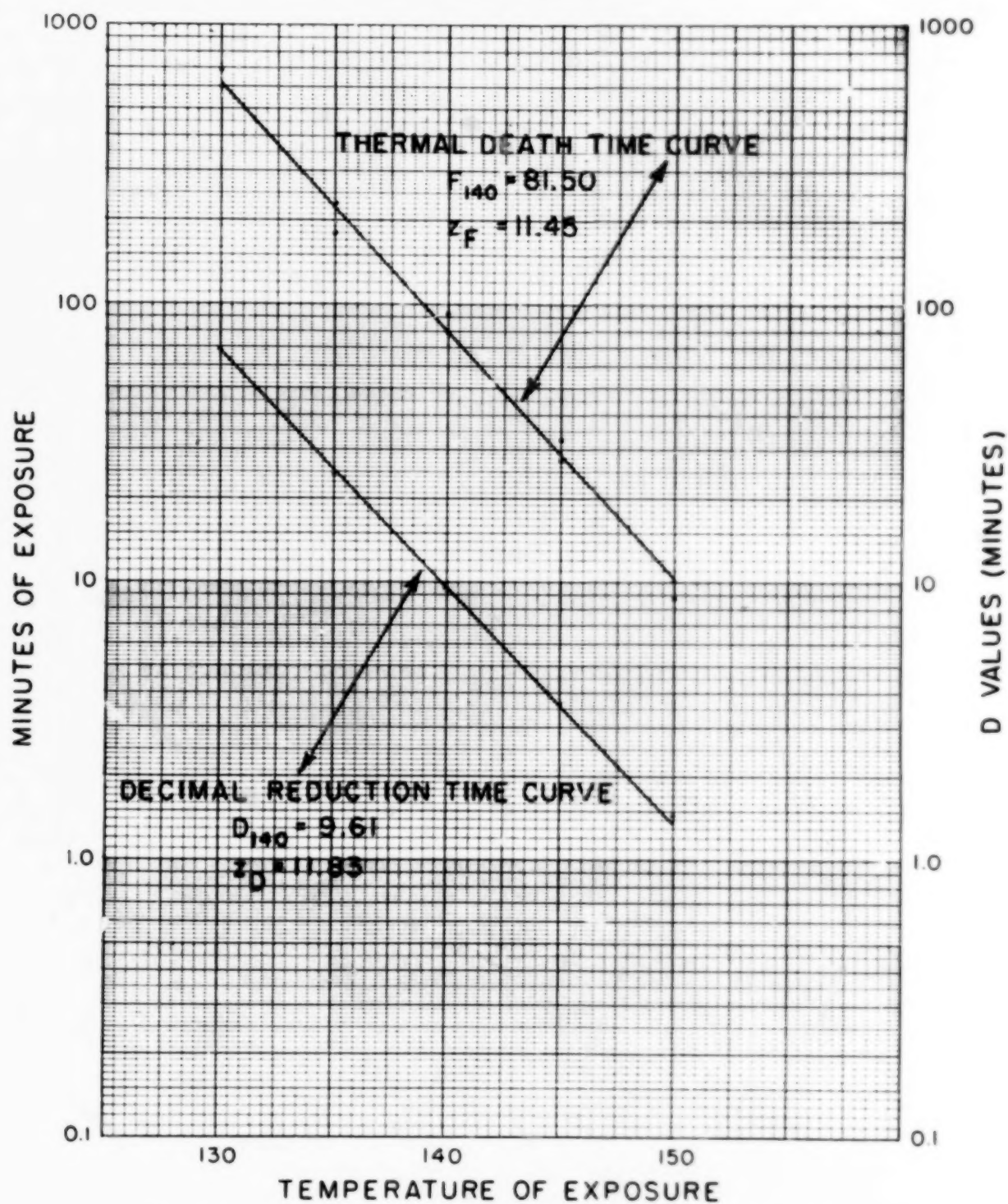


Figure 1. - Heat resistance of *S. senftenberg* 775W in chicken a la king; approximately 1×10^7 cells per tube.

Thermal Death Time Curve

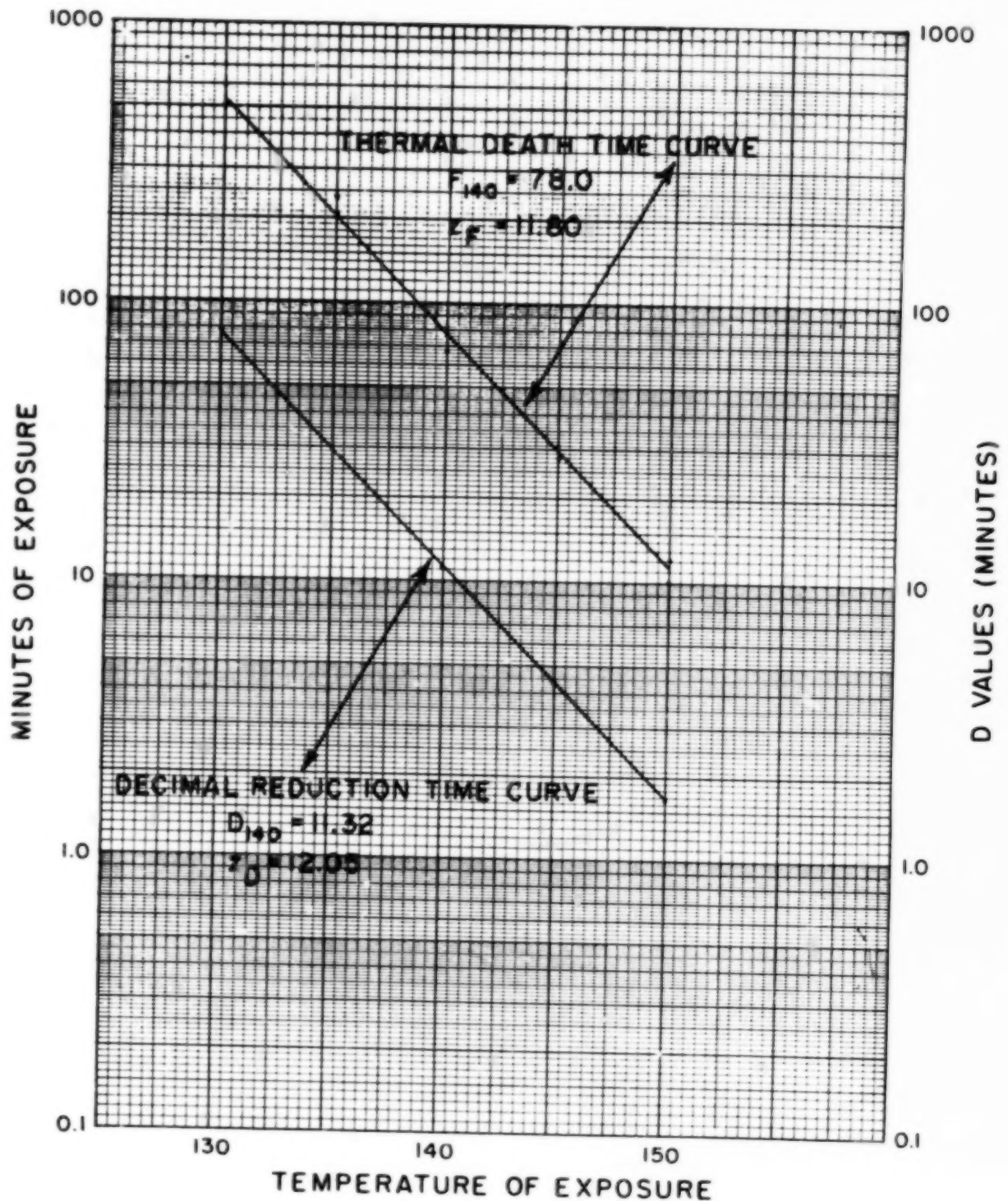


Figure 2. - Heat resistance of *S. senftenbergi* 775W in custard; approximately 1×10^7 cells per tube.

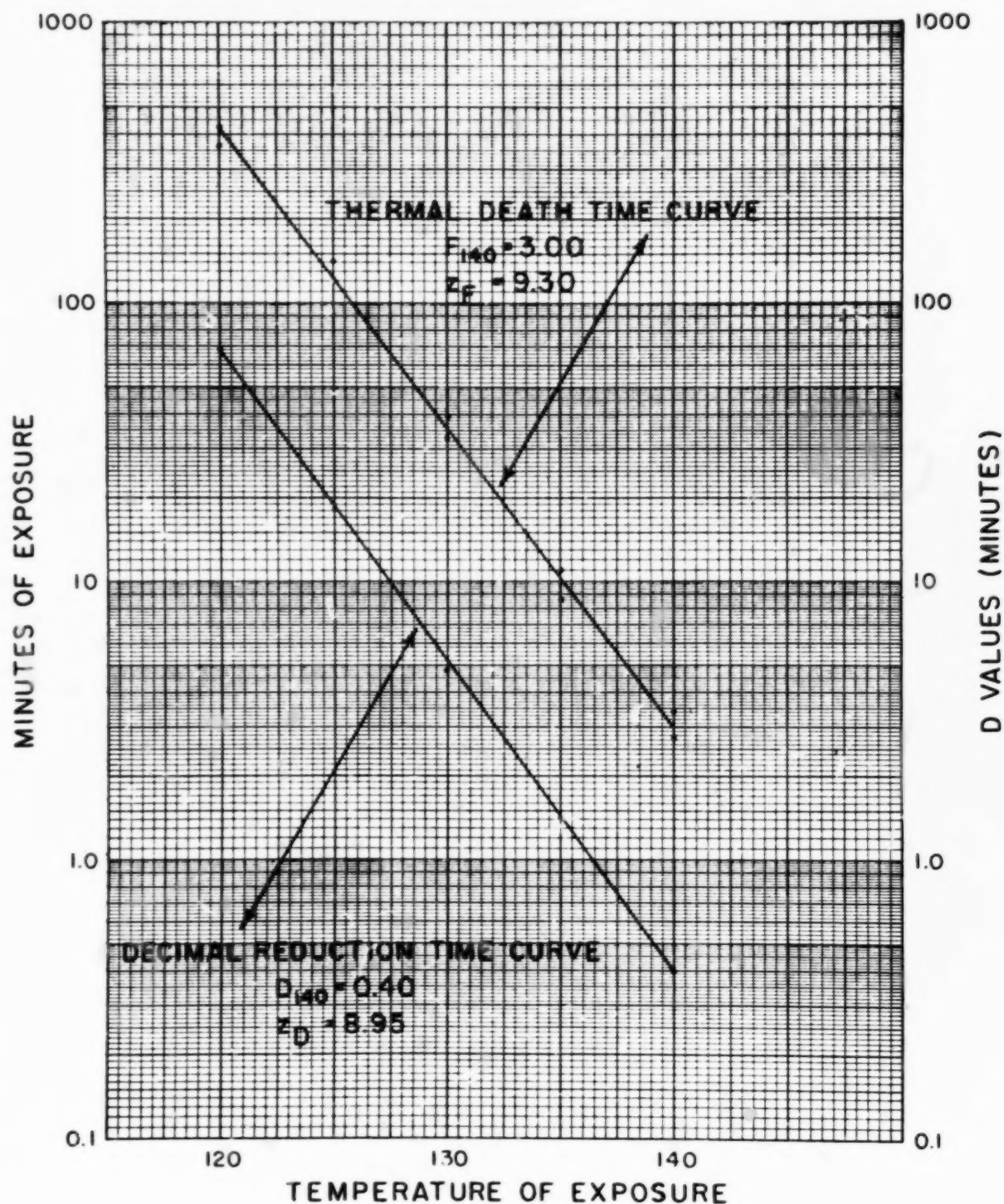


Figure 3. - Heat resistance of *S. manhattan* in chicken à la king; approximately 1×10^7 cells per tube.

Thermal Death Time Curve

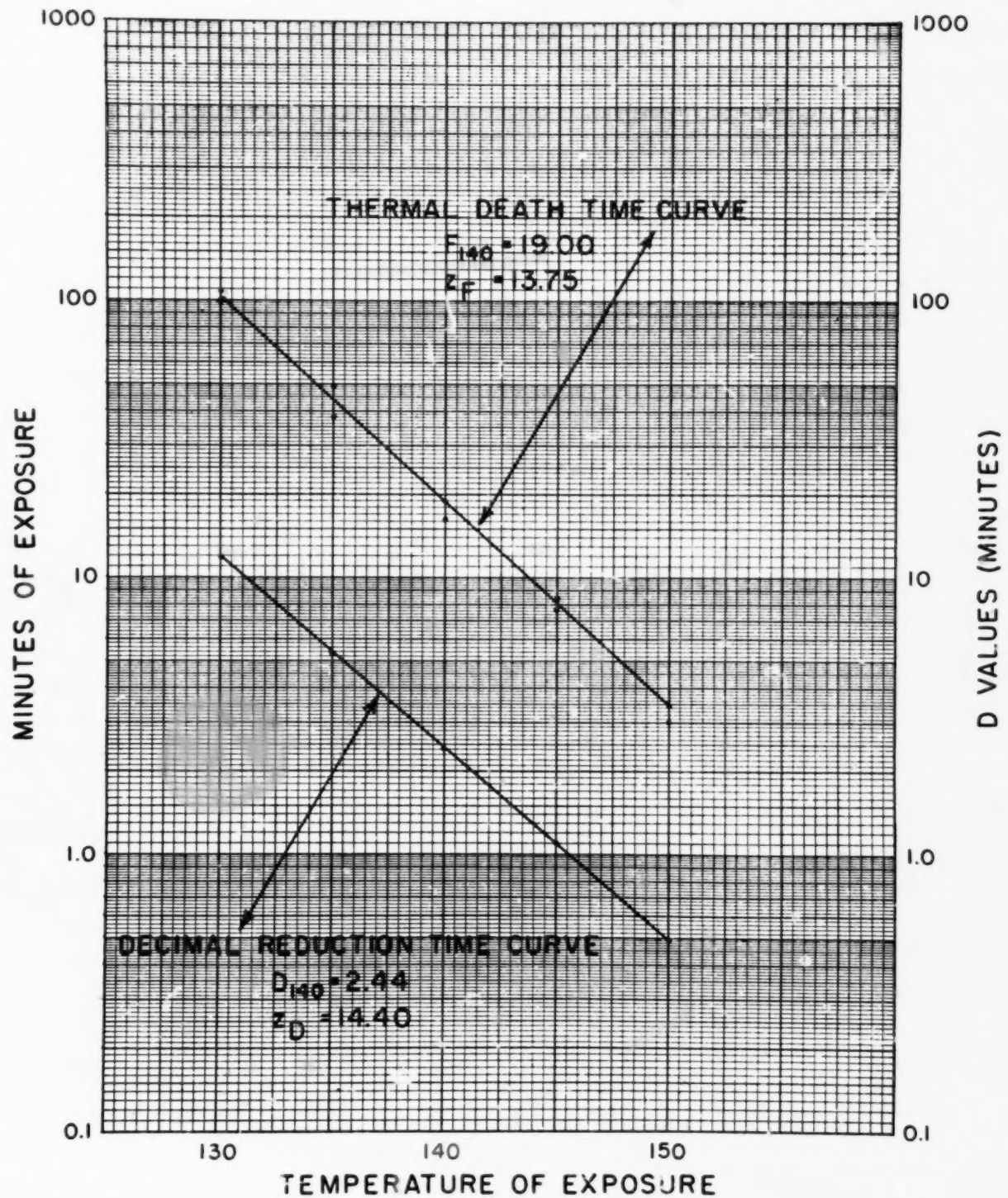


Figure 4 - Heat resistance of *S. manhattan* in custard; approximately 1×10^7 cells per tube.

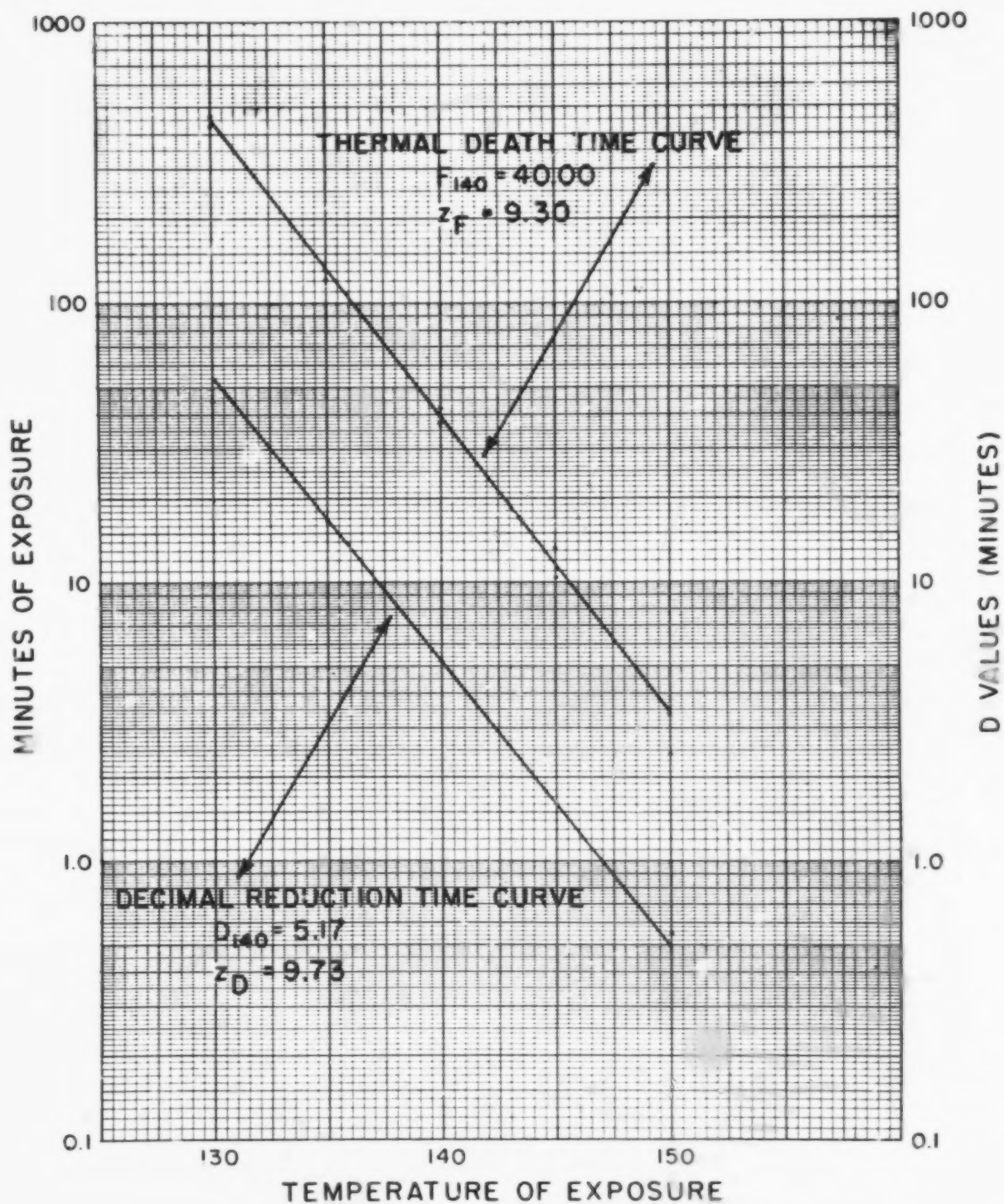


Figure 5. - Heat resistance of *Staph. aureus* Ms149 in chicken à la king; approximately 1×10^7 cells per tube.

Thermal Death Time Curve

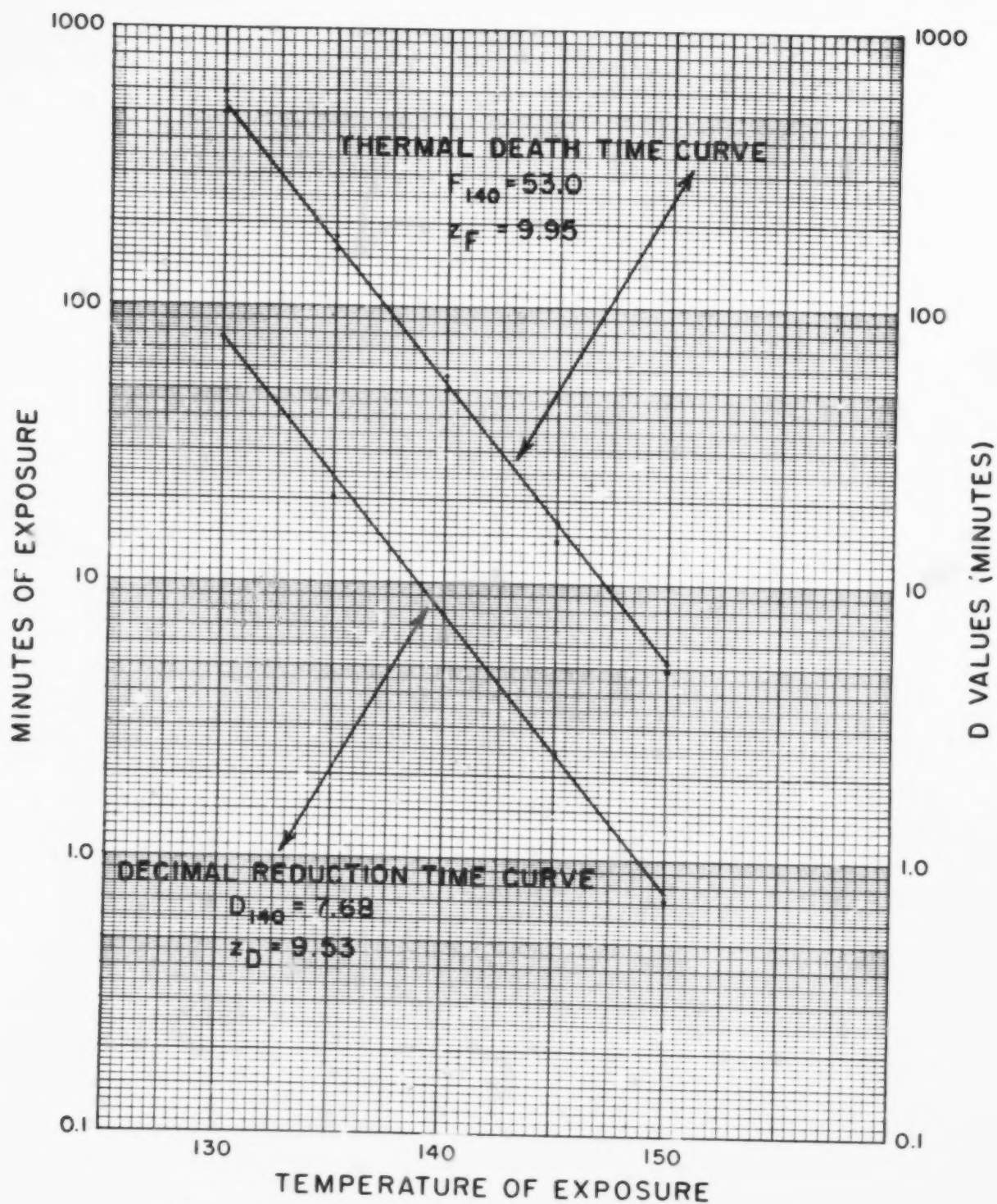


Figure 6. - Heat resistance of *Staph. aureus* Ms149 in custard; approximately 1×10^7 cells per gram.

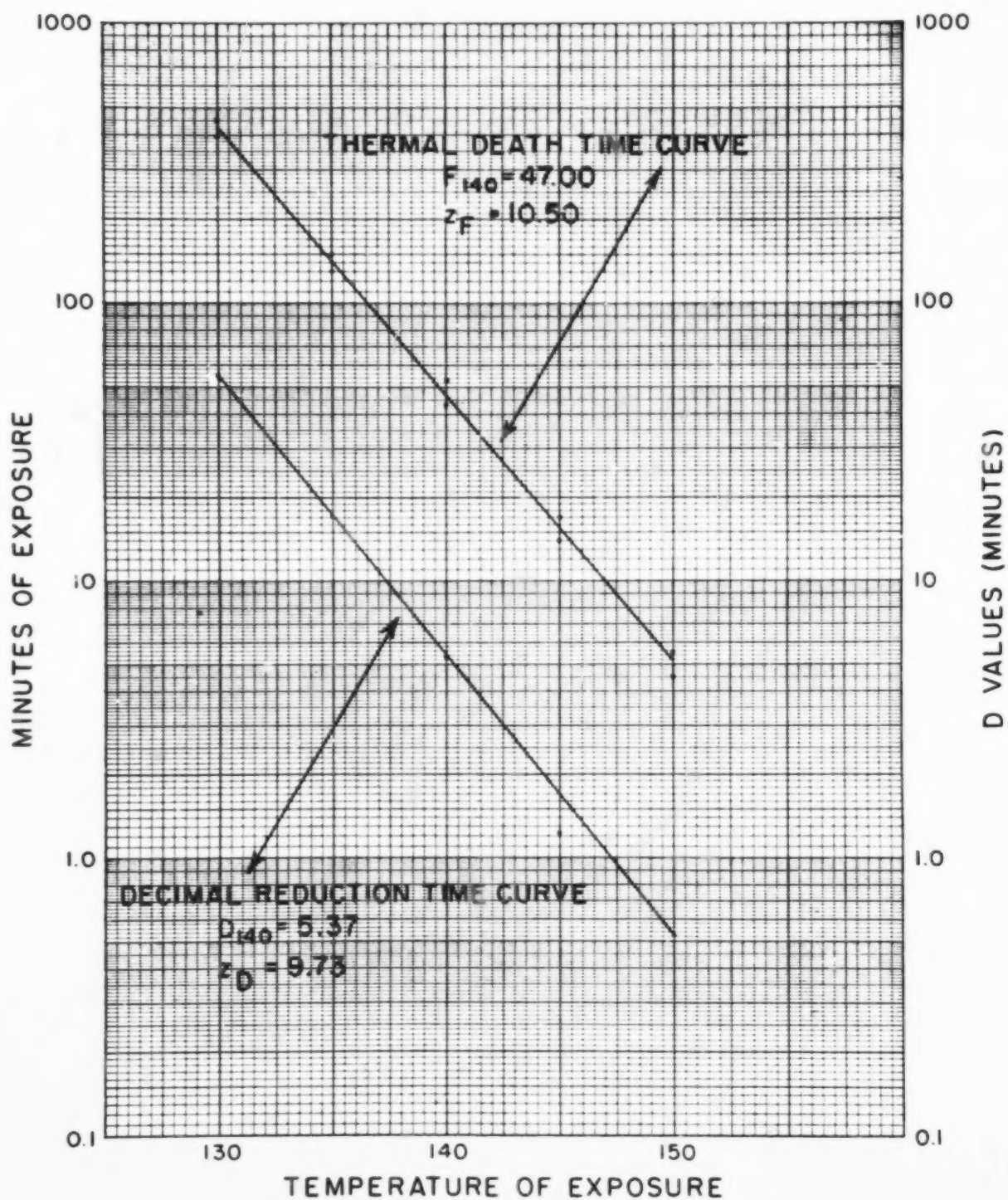


Figure 7. - Heat resistance of *Staph. aureus* 196F in chicken à la king; approximately 1×10^7 cells per tube.

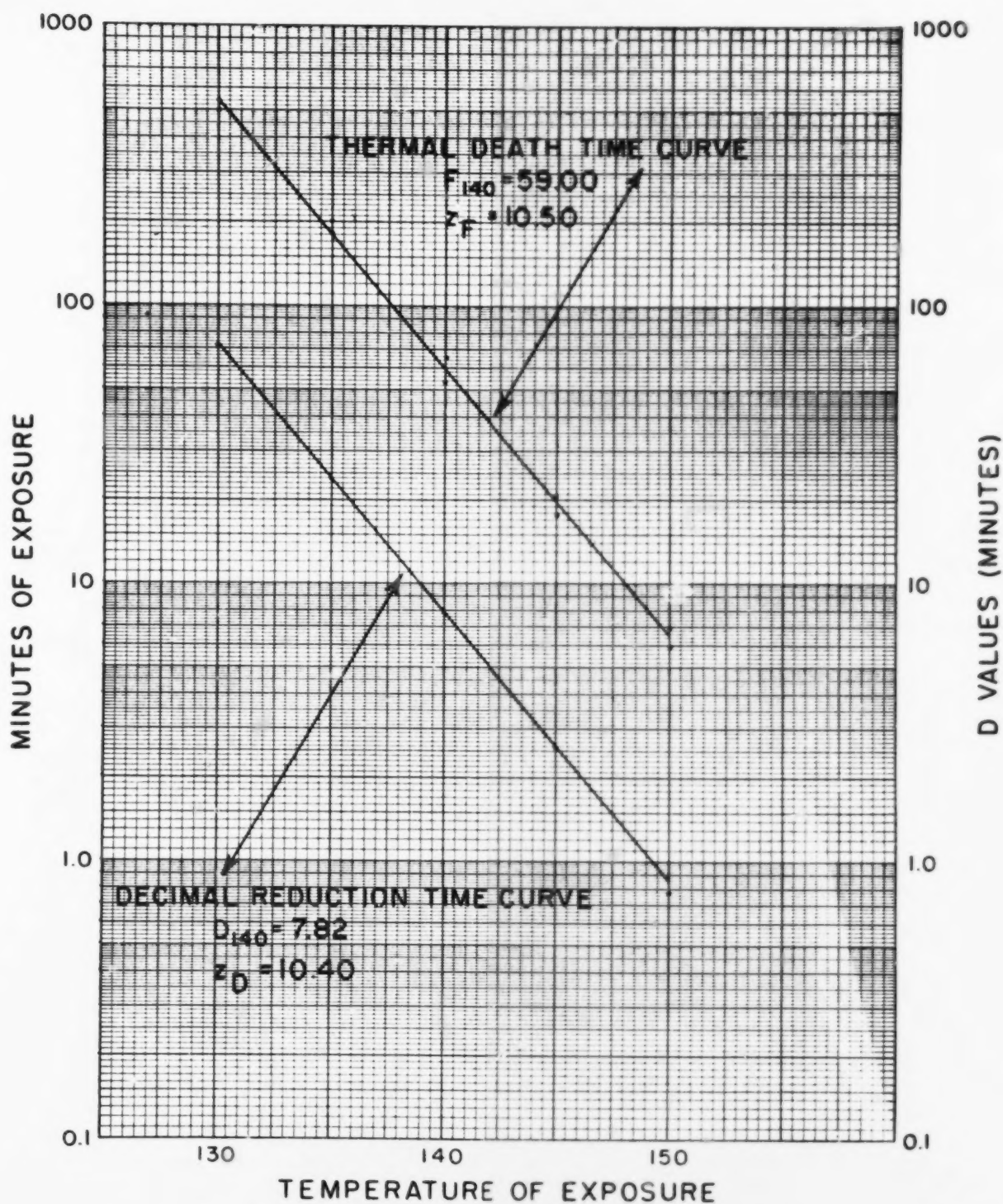


Figure 8. - Heat resistance of *Staph. aureus* 196E in custard; approximately 1×10^6 cells per tube.

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 1. - SURVIVAL OF STAPHYLOCOCCUS AUREUS M_S149 IN CUSTARD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	3	10	0
		4	10	0
		5	2	8
		6	0	10
		7	0	10
		8	0	10
II.	145	12	10	0
		14	10	0
		16	7	3
		18	0	10
		20	2	8
		22	0	10
III.	140	40	10	0
		46	10	0
		52	9	1
		58	2	8
		64	0	10
		70	0	10
IV.	135	60	10	0
		80	10	0
		100	10	0
		120	10	0
		140	10	0
		180	0	10
V.	130	510	0	10
		540	0	10
		600	0	10
		660	0	10
		720	0	10
		780	0	10
VI.	150	3	10	0
		4	10	0
		5	5	5
		6	2	8
		7	0	10
		8	0	10
VII.	140	40	10	0
		48	10	0
		56	2	8
		64	10	10
		72	10	10
		80	10	10
VIII.	135	100	10	0
		140	2	8
		180	0	10
		220	0	10
		260	0	10
		300	0	10

Survival at Various Temperatures

Table 2. - SURVIVAL OF STAPHYLOCOCCUS AUREUS Ms149 IN CHICKEN À LA KING
HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	3	10	0
		4	1	9
		5	0	10
		6	0	10
		7	0	10
		8	0	10
II.	145	9	10	0
		12	2	8
		15	0	10
		18	0	10
		21	0	10
		24	0	10
III.	140	30	10	0
		35	5	5
		40	0	10
		45	0	10
		50	0	10
		55	0	10
IV.	135	100	10	0
		110	7	3
		120	1	9
		130	0	10
		140	0	10
		150	0	10
V.	130	240	10	0
		270	10	0
		300	6	4
		330	1	9
		360	0	10
		390	0	10
VI.	150	3	10	0
		4	4	6
		5	0	10
		6	0	10
		7	0	10
		8	0	10
VII.	140	30	10	0
		36	8	2
		42	2	8
		48	0	10
		54	0	10
		60	0	10
VIII.	130	270	10	0
		300	9	1
		330	8	2
		360	2	8
		390	0	10
		420	0	10

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 3. - SURVIVAL OF STAPHYLOCOCCUS AUREUS 196E IN CUSTARD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure Temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	140	3	10	0
		4	10	0
		5	3	7
		6	1	9
		7	1	9
		8	0	10
II.	145	9	10	0
		12	10	0
		15	10	0
		18	5	5
		21	1	9
		24	0	10
III.	140	30	10	0
		45	10	0
		60	4	6
		75	0	10
		90	0	10
		105	0	10
IV.	135	100	10	0
		130	10	0
		160	5	5
		190	1	9
		220	0	10
		250	0	10
V.	130	450	10	0
		480	10	0
		510	1	9
		540	0	10
		570	0	10
		600	0	10
VI.	150	3.5	10	0
		4.5	10	0
		5.5	7	3
		6.5	1	9
		7.5	1	9
		8.5	0	10
VII.	145	13	10	0
		16	10	0
		19	4	6
		22	1	9
		25	0	10
		28	0	10
VIII.	140	30	10	0
		42	10	0
		54	4	6
		66	0	10
		78	0	10
		90	0	10

Survival at Various Temperatures

Table 4. - SURVIVAL OF STAPHYLOCOCCUS AUREUS 196E IN CHICKEN A LA KING
HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of Exposure	Number of positive tubes	Number of negative tubes
I.	150	2	10	0
		3	9	1
		4	3	7
		5	1	9
		6	1	9
		7	0	10
II.	145	6	10	0
		9	2	8
		12	1	9
		15	0	10
		18	0	10
		21	0	10
III.	140	20	10	0
		30	10	0
		40	2	8
		50	1	9
		60	0	10
		70	0	10
IV.	135	120	10	0
		130	5	5
		140	1	9
		150	0	10
		160	0	10
		170	0	10
V.	130	300	10	0
		350	10	0
		400	6	4
		450	0	10
		500	0	10
		550	0	10
VI.	150	3	10	0
		5	10	0
		7	2	8
		9	0	10
		11	0	10
		13	0	10
VII.	145	6.5	10	0
		9.5	3	7
		12.5	1	9
		15.5	1	9
		18.5	0	10
		21.5	0	10
VIII.	140	25	10	0
		35	8	2
		45	1	9
		55	0	10
		65	0	10
		75	0	10

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 5. - SURVIVAL OF SALMONELLA SENFTENBERG 775W IN CUSTARD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	8	10	0
		10	10	0
		12	8	2
		14	0	10
		16	0	10
		18	0	10
II.	145	20	10	0
		25	10	0
		30	10	0
		35	3	7
		40	2	8
		45	0	10
III.	140	50	10	0
		60	9	1
		70	3	7
		80	0	10
		90	0	10
		100	0	10
IV.	135	150	8	2
		180	3	7
		210	1	9
		240	0	10
		270	1	9
		300	0	10
V.	130	200	10	0
		300	10	0
		400	10	0
		500	9	1
		600	0	10
		700	0	10
VI.	150	6	10	0
		8	10	0
		10	10	0
		12	4	6
		14	0	10
		16	0	10
VII.	140	55	10	0
		65	5	5
		75	1	9
		85	0	10
		95	0	10
		105	0	10
VIII.	130	200	10	0
		300	10	0
		400	9	1
		500	5	5
		600	0	10
		700	0	10

Survival at Various Temperatures

Table 6. - SURVIVAL OF SALMONELLA SENFTENBERG 775W IN CHICKEN A LA KING
HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, of	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	6	10	0
		8	10	0
		10	4	6
		12	0	10
		14	0	10
		16	0	10
II.	145	20	10	0
		25	10	0
		30	5	5
		35	0	10
		40	0	10
		45	0	10
III.	140	40	10	0
		55	10	0
		70	1	9
		85	2	8
		100	0	10
		115	0	10
IV.	135	90	10	0
		120	10	0
		150	9	1
		180	3	7
		210	0	10
		240	0	10
V.	130	150	2	8
		200	1	9
		250	0	10
		300	0	10
		350	0	10
		400	0	10
VI.	150	7	10	0
		9	10	0
		11	2	8
		13	0	10
		15	0	10
		17	0	10
VII.	140	40	10	0
		50	10	0
		60	10	0
		70	5	5
		80	1	9
		90	0	10
VIII.	130	100	10	0
		150	10	0
		200	10	0
		250	10	0
		300	6	4
		350	0	10

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 7. - SURVIVAL OF SALMONELLA MANHATTAN IN CUSTARD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE).

	Exposure temperature °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	1	10	0
		2	10	0
		3	5	5
		4	0	10
		5	0	10
		6	0	10
II.	145	6	10	0
		7	4	6
		8	1	9
		9	0	10
		10	0	10
		11	0	10
III.	140	10	10	0
		13	10	0
		16	10	0
		19	1	9
		22	0	10
		25	0	10
IV.	135	20	10	0
		30	10	0
		40	8	2
		50	0	10
		60	0	10
		70	0	10
V.	130	60	10	0
		70	10	0
		80	10	0
		90	10	0
		100	5	5
		110	0	10
VI.	150	1.5	10	0
		2.5	8	2
		3.5	5	5
		4.5	0	10
		5.5	0	10
		6.5	0	10
VII.	140	9	10	0
		12	10	0
		15	8	2
		18	1	9
		21	0	10
		24	0	10
VIII	130	60	10	0
		70	10	0
		80	10	0
		90	10	0
		100	5	5
		110	0	10

Survival at Various Temperatures

Table 8. - SURVIVAL OF SALMONELLA MANHATTAN IN CHICKEN À LA KING AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	140	3.0	10	0
		3.5	10	0
		4.0	3	7
		4.5	1	9
		5.0	0	10
		5.5	0	10
II.	135	6	10	0
		9	7	3
		12	0	10
		15	0	10
		18	0	10
		21	0	10
III.	130	20	10	0
		28	7	3
		36	0	10
		44	0	10
		52	0	10
		60	0	10
IV.	125	60	10	0
		80	10	0
		100	10	0
		120	5	5
		140	0	10
		160	0	10
V.	120	180	10	0
		240	10	0
		300	10	0
		360	5	5
		420	0	10
		480	0	10
VI.	140	3.0	10	0
		3.5	10	0
		4.0	3	7
		4.5	0	10
		5.0	0	10
		5.5	0	10
VII.	135	7	10	0
		9	10	0
		11	1	9
		13	0	10
		15	0	10
		17	0	10
VIII.	130	20	10	0
		25	10	0
		30	10	0
		35	8	2
		40	5	5
		45	0	10

TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

Table 9. - SURVIVAL OF SALMONELLA SENFTENBERG 775W IN HAM SALAD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	2.5	10	0
		3.5	10	0
		4.5	2	8
		5.5	0	10
		6.5	0	10
		7.5	0	10
II.	140	22	10	0
		25	10	0
		29	4	6
		32	1	9
		35	0	10
		38	0	10
III.	135	68	10	0
		73	7	3
		78	3	7
		83	0	10
		88	0	10
		93	0	10

Survival at Various Temperatures

Table 10. - SURVIVAL OF STAPHYLOCOCCUS AUREUS Ms149 IN HAM SALAD HELD AT VARIOUS TEMPERATURES (APPROXIMATELY 1×10^7 ORGANISMS PER TUBE)

	Exposure temperature, °F	Minutes of exposure	Number of positive tubes	Number of negative tubes
I.	150	2	10	0
		3	10	0
		4	4	6
		5	2	8
		6	0	10
		7	0	10
II.	140	20	10	0
		25	6	4
		30	1	9
		35	1	9
		40	0	10
		45	0	10
III.	130	125	10	0
		140	10	0
		155	10	0
		170	8	2
		185	6	4
		200	0	10